

GLYCOSYLATED HEMOGLOBIN (GHb)

(Ion Exchange Resin method)

For the quantitative determination of Glycohemoglobin in Blood

REF: 254 001 25 Tests
 REF: 254 002 50 Tests
 REF: 254 003 100 Tests

Background

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.

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 a reliable indicator of the efficacy of therapy.

Assay Principle

Glycosylated hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobins HbA1 (Hb A1a, 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 of 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 glycosylated hemoglobin is determined by measuring absorbances of the ratio of the absorbances of the Glycosylated hemoglobin (Ghb) & the Total hemoglobin fraction (THb) . The ratio of the absorbances of GHb and THb of the control and test is used to calculate the percent GHb of the sample.

Expected values

Normal < 8.0 %
 Good control 8.0 – 9.0 %
 Fair control 9.0 – 10.0 %
 Poor control > 10.0 %

It is recommended that each laboratory establish its own normal range representing its patient population.

Reagents	25 Tests	50 Tests	100 Tests
R1 Ion Exchange Resin (Predispensed Tubes)	25 x 3 ml	50 x 3 ml	100 x 3 ml
R2 Lysing Reagent	1 x 12.5 ml	2 x 12.5 ml	4 X 12.5 ml
C Control (10% GHb)	1 x 1 ml	1 x 1 ml	1 x 1 ml
A Resin Separators	25 Nos	50 Nos	100 Nos

Reagents Preparation Storage and Stability

Contents are stable at 2-8°C till the expiry mentioned on the label. Do not freeze. The Resin separators can be removed on opening the kit and stored at R.T.

The Ion Exchange Resin tubes & the Lysing Reagent are ready to use. Reconstitute the Control with 1 ml of distilled water. Allow to stand for 10 mins with occasional mixing. The reconstituted control is stable for at least 7 days when stored at 2-8°C tightly sealed, and at least 4 weeks when stored at -20°C. Do not thaw and refreeze.

Specimens: Whole blood, preferably fresh collected in EDTA. GHb in whole blood is stable for week at 2-8°C

Procedure

Wavelength : 415 nm (Hg 405 nm)
 Temperature : R.T.
 Light path : 1 cm

A. Hemolysate Preparation

1. Dispense 0.5 ml Lysing Reagent into tubes labeled as Control (C) & Test (T).
2. Add 0.1ml of the reconstituted control & well-mixed blood sample into the appropriately labeled tubes. Mix until complete lysis is evident.
3. Allow to stand for 5 minutes.

B. Glycosylated hemoglobin (GHb) Separation

Remove cap from the Ion-Exchange Resin tubes and label as Control & Test.
Add 0.1 ml of the hemolysate from Step A into the appropriately labeled Ion Exchange Resin tubes.
Insert a resin Separator into each tube so that the rubber sleeve is approximately 1 cm above the liquid level of the resin suspension.
Mix the tubes on a rocker, rotator or a vortex mixer continuously for 5 minutes.
Allow the resin to settle, then push the resin separator into the tubes until the resin is firmly packed.
Pour or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water.

C. Total Hemoglobin (THb) fraction

Dispense 5.0 ml of distilled water into tubes labeled as Control & Test.
Add to it 0.02 ml of hemolysate from Step A into the appropriately labeled tube.
Mix well.
Read each absorbance against distilled water

Calculations

$$\text{Ratio of Control (R}_C\text{)} = \frac{\text{Abs. Control GHb}}{\text{Abs. Control THb}}$$
$$\text{Ratio of Test (R}_T\text{)} = \frac{\text{Abs. Test GHb}}{\text{Abs. Test THb}}$$
$$\text{GHb in \%} = \frac{\text{Ratio of Test (R}_T\text{)}}{\text{Ratio of Control (R}_C\text{)}} \times 10 \text{ (Value of Control)}$$

Linearity

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

Notes

Blood samples with Hemoglobin greater than 18 g/dl should be diluted 1 + 1 with Normal saline before the assay.
Samples from patients with Hemoglobinopathies, decreased red cell survival times, gross lipemia may show incorrect results.
Do not use Ion Exchange Resin tubes in case of turbidity or visible discoloration. Diabetics with metabolic imbalance may have extremely high levels of the labile aldimine form. In such cases the incubation time during hemolysate preparation may be increased to 15 minutes to ensure elimination of this in stable fraction.

References

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