SPECTRUM

IVD

The Creative Approach to Bioscience

Glycosylated Hemoglobin (GHb)
(Ion Exchange Resin method)

REF.	Pack size
255 000	15 Tests
254 001	25 Tests
255 001	50 Tests
255 002	100 Tests

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		

Intended Use

GHb reagent is intended for the in-vitro quantitative, diagnostic determination of Glycohemoglobin in blood

Introduction

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.

Method

Ion Exchange Resin method

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.

Reagents

Reagent 1

Ion Exchange Resin (Predispensed Tubes)

Reagent 2 (Lysing)

Lysing Reagent

Control (10% GHb)

A Resin Separators

Harmful (Xn): R20/21/22: Harmful by inhalation, in contact with skin and if swallowed.

Precautions and Warnings

In case of accident or if you feel unwell, seek medical advice immediately. The amount of cyanide present in one bottle of reagent is appreciably less than the minimum lethal dose for an adult. However, hydrogen cyanide is liberated by acidification. Never allow reagent to come in contact with acid.Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagents preparation, storage and stability

Reagents 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. Once Opened, the reagent is stable for 3 months at specified temperature.

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

Deterioration

Do not use Ion Exchange Resin tubes in case of turbidity or visible discolouration. 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

Specimen collection and preservation

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)
Optical path	1 cm
Temperature	RT

A. Hemolysate Preparation

1.Dispense 0.5 ml Lysing Reagent into tubes labeled as Control (C) and 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

1.Remove cap from the Ion-Exchange Resin tubes and label as Control and Test.
2.Add 0.1 ml of the hemolysate from Step A into the appropriately labeled Ion Exchange Resin tubes.
3.Insert a resin Separator 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 or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water.

C. Total Hemoglobin (THb) fraction

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

Calculation

$$\text{Ratio of Control } (R_C) = \frac{\text{Abs.Control GHb}}{\text{Abs.Control THb}}$$

$$\text{Ratio of Test } (R_T) = \frac{\text{Abs.TestGHb}}{\text{Abs.TestTHb}}$$

$$\text{GHb Ratio \%} = \frac{\text{Ratio of test } (R_T)}{\text{Ratio of Control } (R_C)} \times 10 \text{ (value of Control)}$$

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (%)	5.4	11.7
SD	0.2	0.34
CV%	4.07	2.91

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (%)	5.8	11.4
SD	0.208	0.47
CV%	3.59	4.12

Sensitivity

The sensitivity of this assay is 3%

Linearity

4.0% - 20.0 %(HbA1).

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.

Expected values

	HbA1	HbA1c
Normal	< 8.0 %	< 6.0 %
Good control	8.0 – 9.0 %	6.0 – 6.8 %
Fair control	9.0 – 10.0 %	6.8 – 7.65 %
Poor control	> 10.0 %	> 7.65 %

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

Bibliography

1.Bates,H.M.,Lab Manag.,Vol 16 (Jan.1978)
2. Bunn,H.F., Diabetes 130, 613 (1981).
3.Nathan,D.M.,et al., New Eng.J.Med. 310,341 - 346 (1984).

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

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