GLYCOSYLATED HEMOGLOBIN

INTENDED USE

For the quantitative determination of Glycohemoglobin in Blood.

METHOD

VITRO SCIENT. Ion Exchange Resin method.

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 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 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 maxing period, a filter separator is used to remove the resin from the supernatant. The percent glycosylated hemoglobin is determined by measuring absorbances of the glycosylated hemoglobin (GHb) fraction & the total hemoglobin (THb) fraction. The ratio of the absorbances of the Glycosylated hemoglobin & the Total hemoglobin fraction of the Control and the Test is used to calculate the percent Glycosylated hemoglobin of the sample.

REAGENTS

	10 Tests	25 Tests
Ion Exchange Resin (Predispensed Tubes)	10 x 3 ml	25 x 3 ml
Lysing Reagent	5 ml	12.5 ml
Resin Separators	10 Pieces	25 Pieces

- **REAGENT STORAGE AND STABILITY**

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

SPECIMEN

Whole blood. Preferably fresh & collected in EDTA . GHb in whole blood is reported to be stable for one week at 2- 8 °C

PROCEDUR

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 test (T)and control (C) .
2. Add 0.1 ml of the reconstituted control and well mixed blood sample into the appropriately labelled tubes. Mix unitl complete lysis is evident.
3. Allow to stand for 5 minutes.

B. Glycosylated haemoglobin (GHb) Separation:

1. Remove cap from the Ion-Exchange Resin tubes and label as Test and control.
2. Add 0.1 ml of the haemolysate from step A into the appropriately labelled 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 labelled as Test and Control.
2. Add to it 0.02 ml of hemolysate from Step A into the appropriately labelled tube. Mix well.
3. Read each absorbance against distilled water.

CALCULATION

$$\text{Ratio of Test } (R_T) = \frac{\text{Abs test GHb}}{\text{Abs test THb}}$$

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

$$\text{GHb in \%} = \frac{\text{Ratio of Test (RT)}}{\text{Ratio of Control (RC)}} \times 10 \text{ (Value of Control)}$$

EXPECTED VALUES

	GHbA %	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 %

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

LINEARITY

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

PRECAUTIONS

Blood samples with Hemoglobin greater than 18 g/dl should be diluted 1 + 1 with Normal saline before the assay.

Sample 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 discolouration.

Diabetics with metabolic imbalance may have extremely high levels of the labile aldimine from. In such cases the incubation time during hemolysate preparation may be increased to 15 minutes to ensure elimination of this instable fraction.

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Article # :153 -EN
Date of Revision : 10/2012

CE

CONVERSIONS

Table for the conversion of Glycosylated Hemoglobin A1 (GHbA1) to Gylcosylated Hemoglobin A1c (HbA1c) and to the Mean Blood Glucose level (MBG).

GHbA1	HbA1c	MBG
5.0	3.46	---
5.1	3.54	---
5.2	3.63	---
5.3	3.71	---
5.4	3.79	---
5.5	3.88	---
5.6	3.96	---
5.7	4.04	---
5.8	4.13	---
5.9	4.21	---
6.0	4.30	57
6.1	4.38	60
6.2	4.46	63
6.3	4.55	65
6.4	4.63	68
6.5	4.71	71
6.6	4.80	74
6.7	4.88	77
6.8	4.97	79
6.9	5.05	82
7.0	5.13	85
7.1	5.22	88
7.2	5.30	91
7.3	5.39	93
7.4	5.47	96
7.5	5.55	99
7.6	5.64	102
7.7	5.72	104
7.8	5.80	107
7.9	5.89	110
8.0	5.97	113
8.1	6.06	116
8.2	6.14	118
8.3	6.22	121
8.4	6.31	124
8.5	6.39	127
8.6	6.47	130
8.7	6.56	132
8.8	6.64	135
8.9	6.73	138
9.0	6.81	141
9.1	6.89	144
9.2	6.98	146
9.3	7.06	149
9.4	7.15	152
9.5	7.23	155
9.6	7.31	158
9.7	7.40	160
9.8	7.48	163
9.9	7.56	166
10.0	7.65	169
10.1	7.73	171
10.2	7.82	174
10.3	7.90	177
10.4	7.98	180
10.5	8.07	183
10.6	8.15	185
10.7	8.23	188
10.8	8.32	191
10.9	8.40	194
11.0	8.49	197
11.1	8.57	199
11.2	8.65	202
11.3	8.74	205
11.4	8.82	208
11.5	8.91	211
11.6	8.99	213
11.7	9.07	216
11.8	9.16	219
11.9	9.24	222
12.0	9.32	224
12.1	9.41	227
12.2	9.49	230
12.3	9.58	233
12.4	9.66	236
12.5	9.74	238
12.6	9.83	241
12.7	9.91	244
12.8	9.99	247
12.9	10.08	250
13.0	10.16	252
13.1	10.25	255
13.2	10.33	258
13.3	10.41	261
13.4	10.50	264
13.5	10.58	266
13.6	10.66	269
13.7	10.75	272
13.8	10.83	275
13.9	10.92	278
14.0	11.00	280
14.1	11.08	---
14.2	11.17	---
14.3	11.25	---
14.4	11.34	---
14.5	11.42	---
14.6	11.50	---
14.7	11.59	---
14.8	11.67	---
14.9	11.75	---
15.0	11.84	---
15.1	11.92	---
15.2	12.01	---
15.3	12.09	---
15.4	12.17	---
15.5	12.26	---
15.6	12.34	---
15.7	12.42	---
15.8	12.51	---
15.9	12.59	---
16.0	12.68	---
16.1	12.76	---
16.2	12.84	---
16.3	12.93	---
16.4	13.01	---
16.5	13.09	---
16.6	13.18	---
16.7	13.26	---
16.8	13.35	---
16.9	13.43	---
17.0	13.51	---
17.1	13.60	---
17.2	13.68	---
17.3	13.77	---
17.4	13.85	---
17.5	13.93	---
17.6	14.02	---
17.7	14.10	---
17.8	14.18	---
17.9	14.27	---
18.0	14.35	---
18.1	14.44	---
18.2	14.52	---
18.3	14.60	---
18.4	14.69	---
18.5	14.77	---
18.6	14.85	---
18.7	14.94	---
18.8	15.02	---
18.9	15.11	---
19.0	15.19	---
19.1	15.27	---
19.2	15.36	---
19.3	15.44	---
19.4	15.53	---
19.5	15.61	---
19.6	15.69	---
19.7	15.78	---
19.8	15.86	---
19.9	15.94	---
20.0	16.03	---

In the test study done by Nathan , D.M . et. al . they calculated the Mean Blood Glucose concentration from the value of Hba1% measured with the equation

MBG in mg/dl =
33.3 x HbA1c value - 86

These values are linear in the range of 6.5 - 13% of HbA1 c values

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Article # :153 -EN
Date of Revision : 10/2012

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