N BIO - Glyco Hemoglobin

(Ion exchange resin method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - GHB	10 Tests	DGHB00010T
N BIO - GHB	30 Tests	DGHB00030T

GENUINE BIOSYSTEM

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 PRINCIPLE

Glycosylated hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobin's HbA1 (HbA1a, A1b, Alc) 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 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.

KIT CONTENTS

Reagent Name	DGHB00010T	DGHB00030T
R1 Predispensed tube	10 No's	30 No's
R Lysing Reagent	1 x 5 ml	1 x 15 ml
R3 Resin Separators	10 No's	30 No's

I WORKING REAGENT PREPARATION AND STABILITY

Contents 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.

WARNINGS AND NOTES

Handle with same precautions used for all human blood samples.

- No special additives or preservatives other than anticoagulants are required.
- Gross lipemia may cause falsely high results. For grossly lipemic samples centrifugate the red cells and remove the lipemic plasma replacing it with an approximately equal amount of saline and proceed with performances of test. Glycosylated HbS and HbC bind lightly to the resin which produces falsely low results.

Fetal hemoglobin (HbF) does not interfere significantly in the assay. the unstable fraction (aldimine) is eliminated during resin mixing and does not contribute to glycohemoglobin value.

-Glycohemoglobin in the sample is stable for 7 days at 2-8°C

SPECIMEN

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

PROCEDURE

Wavelength	415nm (Hg 405 nm)
Temperature	R.T
Cuvette	1 cm

Step A. Hemolysate Preparation

1. Dispense 250 ul Lysing Reagent into required number of labelled tubes for different samples.
2. Add 50 ul well mixed blood sample into the approximately labelled tubes and mix well.
3. Incubate for 5 minutes at R.T to allow complete lysis of R.B.C

Step B. Glycosylated Hemoglobin (GHb) Separation:

1. Remove cap from the Ion-Exchange Resin tubes and label the tubes as required samples.
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 minuets.
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 at 415 nm (405-420 nm).

Step C. Total Hemoglobin (THb) fraction

1) Dispense 5.0 ml of distilled water into tubes labeled as required samples.
2) Add to it 0.02 ml of hemolysate from Step A in to the appropriately labeled tube.
3) Mix well
4) Read each absorbance against distilled water at 415 nm (405-420 nm).

CALCULATION

Results for the unknown samples are calculated as follows:

$$\text{GHB in \%} = \frac{\text{Abs. of GHB}}{\text{Abs. of THB}} \times 4.61 \quad \text{(Assay Factor)}$$

REFERENCE VALUE

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.

QUALITY CONTROL

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

PERFORMANCE CHARACTERISTICS

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 instable fraction.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

Trivelli, L.A., Ranney, H.M. and Lai, H.T., New Eng. J. med. 284, 353 (1971).

Nathan, D.M.., et al., New Eng. J. med. 310, 341-346 (1984)

Bunn, H.F., Diabetes 130, 613 (1981)

Bates, H.M., Lab Manag., Vol 16 (Jan 1978)

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	GHbA1	HbA1C	MBG
5.0	3.46				
5.1	3.54		8.4	6.31	124
5.2	3.63		8.5	6.39	127
5.3	3.71		8.6	6.47	130
5.4	3.79		8.7	6.56	132
5.5	3.88		8.8	6.64	138
5.6	3.96		9.0	6.81	141
5.7	4.04		9.1	6.89	144
5.8	4.13		9.2	6.98	146
5.9	4.21		9.3	7.06	149
6.0	4.30	57	9.4	7.15	152
6.1	4.38	61	9.5	7.23	155
6.2	4.46	63	9.6	7.31	158
6.3	4.55	65	9.7	7.40	160
6.4	4.63	68	9.8	7.48	163
6.5	4.71	71	9.9	7.56	166
6.6	4.80	74	10.0	7.65	169
6.7	4.88	77	10.1	7.73	171
6.8	4.97	7	10.2	7.82	174
6.9	5.05	82	10.3	7.90	177
7.0	5.13	85	10.4	7.98	180
7.1	5.22	88	10.5	8.07	183
7.2	5.30	91	10.6	8.15	185
7.3	5.39	93	10.7	8.23	188
7.4	5.47	96	10.8	8.32	191
7.5	5.55	99	10.9	8.40	194
7.6	5.64	102	11.0	8.49	197
7.7	5.72	104	11.1	8.57	199
7.8	5.80	107	11.2	8.65	202
7.9	5.89	110	11.3	8.74	205
8.0	5.97	113	11.4	8.82	208
8.1	6.06	116	11.5	8.91	211
8.2	6.14	118	11.6	8.99	213
8.3	6.22	121	11.7	9.07	216
11.8	9.16	219	15.9	12.59	-
11.9	9.24	222	16.0	12.68	-
12.0	9.32	224	16.1	12.76	-
12.1	9.41	227	16.2	12.84	-
12.2	9.49	230	16.3	12.93	-
12.3	9.58	233	16.4	13.01	-
12.4	9.66	236	16.5	13.09	-
12.5	9.74	238	16.6	13.18	-
12.6	9.83	241	16.7	13.26	-
12.7	9.91	244	16.8	13.35	-
12.8	9.99	247	16.9	13.43	-
12.9	10.08	250	17.0	13.51	-
13.0	10.16	252	17.1	13.60	-
13.1	10.25	255	17.2	13.68	-
13.2	10.33	258	17.3	13.77	-
13.3	10.41	261	17.4	13.85	-
13.4	10.50	264	17.5	13.93	-
13.5	10.58	266	17.6	14.02	-
13.6	10.66	269	17.7	14.10	-
13.7	10.75	272	17.8	14.18	-
13.8	10.83	275	17.9	14.27	-
13.9	10.92	278	18.0	14.35	-
14.0	11.00	280	18.1	14.44	-
14.1	11.08		18.2	14.52	-
14.2	11.17		18.3	14.60	-
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				

In the test study done by Nathan , D.M. et.al they calculated the Mean Blood Glucose concentration from the value of HbA1c % measure 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 HbA1c values.

IVD CE

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GBPL/DGHB/04 12.20