NS BIOTEC

GLYCOSYLATED HEMOGLOBIN KIT

(Ion Exchange Resin method)
For the quantitative determination of Glycohemoglobin in Blood
(For invitro Diagnostic use only)

REF. GLY-M- 20 (20 test)
GLY-M- 10 (10 test)
GLY-M- 25 (25 test)

SUMMARY

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.

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

LINEARITY

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

Normal reference 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.

Contents	**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 Nos	25 Nos

Storage / 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.

Sample material

Whole blood. Preferably fresh & collected in EDTA . GHb in whole blood is reported to be stable for one 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 test (T) .
2. Add 0.1 ml of the reconstituted well mixed blood sample into the appropriately
labeled tubes. Mix unit 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.
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.
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

CALCULATIONS

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

GHbA in % = Ratio of Test (R_T) x 10

Notes

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 lon 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.

REFERENCES

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).

	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By

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GHbA1	HbA1c	GHbA1	HbA1c	GHbA1	HbA1c	GHbA1	HbA1c
5.0	3.46	9.0	6.81	13.0	10.16	17.0	13.51
5.1	3.54	9.1	6.89	13.1	10.25	17.1	13.60
5.2	3.63	9.2	6.98	13.2	10.33	17.2	13.68
5.3	3.71	9.3	7.06	13.3	10.41	17.3	13.77
5.4	3.79	9.4	7.15	13.4	10.50	17.4	13.85
5.5	3.88	9.5	7.23	13.5	10.58	17.5	13.93
5.6	3.96	9.6	7.31	13.6	10.66	17.6	14.02
5.7	4.04	9.7	7.40	13.7	10.75	17.7	14.10
5.8	4.13	9.8	7.48	13.8	10.83	17.8	14.18
5.9	4.21	9.9	7.56	13.9	10.92	17.9	14.27
6.0	4.30	10.0	7.65	14.0	11.00	18.0	14.35
6.1	4.38	10.1	7.73	14.1	11.08	18.1	14.44
6.2	4.46	10.2	7.82	14.2	11.17	18.2	14.52
6.3	4.55	10.3	7.90	14.3	11.25	18.3	14.60
6.4	4.63	10.4	7.98	14.4	11.34	18.4	14.69
6.5	4.71	10.5	8.07	14.5	11.42	18.5	14.77
6.6	4.80	10.6	8.15	14.6	11.50	18.6	14.85
6.7	4.88	10.7	8.23	14.7	11.59	18.7	14.94
6.8	4.97	10.8	8.32	14.8	11.67	18.8	15.02
6.9	5.05	10.9	8.40	14.9	11.75	18.9	15.11
7.0	5.13	11.0	8.49	15.0	11.84	19.0	15.19
7.1	5.22	11.1	8.57	15.1	11.92	19.1	15.27
7.2	5.30	11.2	8.65	15.2	12.01	19.2	15.36
7.3	5.39	11.3	8.74	15.3	12.09	19.3	15.44
7.4	5.47	11.4	8.82	15.4	12.17	19.4	15.53
7.5	5.55	11.5	8.91	15.5	12.26	19.5	15.61
7.6	5.64	11.6	8.99	15.6	12.34	19.6	15.69
7.7	5.72	11.7	9.07	15.7	12.42	19.7	15.78
7.8	5.80	11.8	9.16	15.8	12.51	19.8	15.86
7.9	5.89	11.9	9.24	15.9	12.59	19.9	15.94
8.0	5.97	12.0	9.32	16.0	12.68	20.0	16.03
8.1	6.06	12.1	9.41	16.1	12.76		
8.2	6.14	12.2	9.49	16.2	12.84		
8.3	6.22	12.3	9.58	16.3	12.93		
8.4	6.31	12.4	9.66	16.4	13.01		
8.5	6.39	12.5	9.74	16.5	13.09		
8.6	6.47	12.6	9.83	16.6	13.18		
8.7	6.56	12.7	9.91	16.7	13.26		
8.8	6.64	12.8	9.99	16.8	13.35		
8.9	6.73	12.9	10.08	16.9	13.43		

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 =33.3x HbA1c value -86
These values are linear in the range of 6.5-13% of HbA1 c values.