Glycosylated Hemo-

(Ion Exchange Resin Method)

Code : 10009/10/11 (10 / 20 / 50 Tests)

GLYCOSYLATED HEMOGLOBIN kit is intended for the In Vitro Quantitative determination of percent Glycosylated Hemoglobin (GHb%) in human whole blood.

SUMMARY & EXPLANATION OF TEST :

Glycosylated Hemoglobin (GHb) is a normal adult hemoglobin (HbA_1) which is covalently bonded to a glucose molecule. GHb concentration is dependent on the average blood glucose concentration. It is formed progressively and irreversibly over a period of time and is stable till the life of the RBC.

A Single glucose determination gives a value which is true only at the time the blood sample is drawn. GHb on the other hand is unaffected by diet, insulin or exercise on the day of testing and thus reflects the average glucose level over the last several weeks. Hence, it reflects on the long term metabolic control of glucose in individuals. GHb is now widely recognised as an important test for the diagnosis of Diabetes mellitus and is a reliable indicator of the efficacy of therapy. Abraham et al reported excellent correlation between HbA_1 concentration and diabetic control and concluded that the determination of HbA_1 rather than HbA_{1c} (a fraction of HbA_1) be used for clinical purpose.

There are several acceptable methods of GHb measurement like electrophoresis, ion-exchange chromatography, affinity chromatography, HPLC and colorimetry. Excel's GHb kit based upon the property of non-glycosylated hemoglobin to bind with a weak cation exchange resin leaving GHb free in the supernatant.

PRINCIPLE :

Whole blood is mixed with lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation-exchange resin. the non-glycosylated hemoglobin binds to the resin leaving GHb free in the supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and of the total Hb.

REAGENTS & ACCESSORIES PROVIDED :

		10 Tests	20 Tests	50 Tests
1.	Resin Tubes	10 x 3 ml	20 x 3 ml	50 x 3 ml
2.	Lysing Reagent	5 ml	10 ml	2 x 10 ml
3.	Control	0.5 ml	0.5 ml	0.5 ml
4.	Resin Separators	10 Nos	20 Nos	50 Nos

All reagents are stable at 2-8^{o}C till the expiry date mentioned on the label. Do not freeze.

SPECIMEN :

Whole blood collected with EDTA/Heparin. GHb in blood is found to be stable for one week at 2-8^{o}C

REAGENT PREPARATION :

Dissolve the control (3) with 0.5 ml of deionized water by inverting / swirling. Do not shake vigorously. Reconstituted control is stable for 3 days at 2-8^{o}C or 21 days at - 20^{o}C.

PRECAUTIONS :

1. Ion exchange Resin is prepipetted and securely sealed to ensure accuracy and reproducibility of results. Do not use resin Tube in case of visible and significant leakage.
2. Ensure constant assay temperature (preferable 23^{o}C) of Resin during step II of the assay.
3. Initial use of control is advised to check test system performance within limits.

EXPECTED RANGE :

Non Diabetic	:	4.5 to 8.0 %
Good Control	:	8.0 to 9.0 %
Fair Control	:	9.0 to 10.0 %
Poor Control	:	10.0 and above

LINEARITY : This method is linear upto 20%

PROCEDURE :

Assay temperature	:	23$\pm$1^{o}C or 30$\pm$1^{o}C
Wave length	:	415 nm

Step I - Hemolysate preparation

1. Pipette 0.25 ml of lysing reagent (2) in a test tube.
2. Add to it 0.05 ml of well mixed sample/control.
3. Mix well and allow to stand at room temperature for 5 minutes.

Step II - GHb separation and assay

1. Bring a Resin Tube (1) to assay temperature by incubating the tube in water bath.
2. Add to it 0.1 ml of hemolysate (from step 1)
3. Position a Resin Separator in the tube, so that the rubber sleeve is approximately 3 cms, above the resin level.
4. Mix the contents on vortex mixer continuously for 5 minutes
5. Allow the Resin to settle at assay temperature for 5 minutes push down the Resin separator in the tube until the Resin is firmly packed.
6. Pour the supernatant directly into a cuvette and measure the absorbance against deionized water.

Step III - Total Hemoglobin (THb) assay

1. Pipette 5.0 ml of deionized water into a test tube.
2. Add to it 0.02 ml of hemolysate (from step 1).
3. Mix and read absorbance against deionized water.

CALCULATION :

$$GHb\% = \frac{\text{A of GHb}}{\text{A of THb}} \times 10 \times \text{Temp. factor (Tf)}$$

For assay at 23^{o}C Tf = 1.0; at 30^{o}C Tf = 0.9

NOTES :

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY :

1. Trivelli, LIA. et al (1971) New Eng. J. Med. 284:353.
2. Gonen, B (1978) Diabetologia 15:1
3. Gabby, K.H. et al (1977) J.Clin. End. Met. 44:859
4. Abraham, E.c. et al (1978) Diabetes 27:931.
5. Bunn, H.F. (1981) Diabetes 30:613.

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CONVERSION CHART OF GLYCOSYLATED HEMOGLOBIN A1% TO MEAN BLOOD GLUCOSE AND GLYCOSYLATED HEMOGLOBIN A1c%

The glycosylated hemoglobin assay has been validated as a reliable indicator of mean blood glucose (MBG) levels for a period of 8-12 week period prior to determination. This assay provides valuable information for the physician's clinical assessment of long term diabetic control. Physicians have conventionally used information such as symptoms, urine tests and random blood glucose determination to evaluate the metabolic state of their diabetic patient and to estimate roughly the average blood glucose of the patient. Recently, the glycohemoglobin test has been shown to have a linear correlation with MBG results from patients performing frequent self-monitoring of blood glucose levels.

Using this correlation, a table of the glycosylated Hemoglobin A1% from the Glycosylated Hemoglobin assay A1c% & Mean Blood Glucose is obtained.

A1	A1c	MBG	A1	A1c	MBG
6.0	4.30	35	9.9	7.56	178
6.1	4.38	39	10.0	7.64	182
6.2	4.46	43	10.1	7.73	186
6.3	4.54	46	10.2	7.81	189
6.4	4.63	50	10.3	7.89	193
6.5	4.71	54	10.4	7.98	197
6.6	4.79	58	10.5	8.06	200
6.7	4.88	61	10.6	8.15	204
6.8	4.96	65	10.7	8.23	207
6.9	5.05	68	10.8	8.31	211
7.0	5.13	72	10.9	8.40	215
7.1	5.21	76	11.0	8.48	219
7.2	5.30	79	11.1	8.56	222
7.3	5.38	83	11.2	8.65	226
7.4	5.46	87	11.3	8.73	230
7.5	5.55	90	11.4	8.82	233
7.6	5.63	94	11.5	8.90	237
7.7	5.72	98	11.6	8.98	241
7.8	5.80	101	11.7	9.07	244
7.9	5.88	105	11.8	9.15	248
8.0	5.97	109	11.9	9.24	252
8.1	6.05	112	12.0	9.32	255
8.2	6.14	116	12.1	9.40	259
8.3	6.22	120	12.2	9.49	263
8.4	6.30	123	12.3	9.57	266
8.5	6.39	127	12.4	9.65	270
8.6	6.47	131	12.5	9.74	274
8.7	6.55	134	12.6	9.82	277
8.8	6.64	138	12.7	9.91	281
8.9	6.72	142	12.8	9.99	285
9.0	6.81	145	12.9	10.07	288
9.1	6.89	149	13.0	10.16	292
9.2	6.97	153	13.1	10.24	295
9.3	7.06	156	13.2	10.33	299
9.4	7.14	160	13.3	10.41	304
9.5	7.22	164	13.4	10.49	309
9.6	7.31	167	13.5	10.58	314
9.7	7.39	171	13.6	10.66	320
9.8	7.48	175	13.7	10.74	326

It should be noted that glycosylated hemoglobin values below 6.0% may indicate the presence of abnormal hemoglobins (S and C) of hemolytic disorders. Additional tests are the recommended.

REFERENCES :

(1) Nathan, D.M., et al., The Clinical information value of the Glycosylated Assay, The New England Journal of Medicine, 310 : 346 (1984).