GLYCOSYLATED HEMOGLOBIN Ion Exchange

Diagnostic reagent for the in-vitro quantitative determination of percent Glycosylated Hemoglobin (GHb%) in human whole blood using Ion Exchange Resin method.

REF: V/GL01.010	10 test	REF: V/GL01.050	50 test
REF: V/GL01.025	25 test		

CLINICAL SIGNIFICANCE

Glycosylated Hemoglobin (GHb) is a normal adult hemoglobin (HbA1) 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. This process is slow, non-enzymatic and is dependent on the average blood glucose concentration over a period of time. 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 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 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 hemolyzed 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 toremove the resin from the supernatant. The percent glycosylated hemoglobin is determined by measuring absorbances of 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

Reagent (R):	**Composition**
R1: Lysing Reagent	Lysis buffer for blood
R2: Control	10% GHb.

REAGENT COMPOSITION

- Ion Exchange Resin (Pre-dispensed Tubes)
- Resin Separators

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.
Good Laboratories practices using appropriate precautions should be followed in: Wearing personnel protective equipment (overall, gloves, glasses,..).
Do not pipette by mouth.
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.
Respect country requirement for waste disposal..
S56: dispose of this material and its container at hazardous orspecial waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.
For further information, refer to the **Lab.Vie**. glycosylated hemoglobin
reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. GHb reagent is stable until expiration date stated on label when properly stored refrigerated at 2-8°C (do not freeze). The Resin separators can be removed on opening the kit and stored at room temperature.
The ion exchange resin tubes and 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.

Deterioration
The **Lab.Vie**. Ion Exchange Resin is pre-pipetted and securely sealed to ensure accuracy and reproducibility of results. Do not use resin Tube in case of visible turbidity or visible discoloration and significant leakage.

SPECIMEN COLLECTION AND PRESERVATION

Whole Blood
Specimen preferably fresh and collected in EDTA. The stability of GHB in whole blood is reported to be for one week at 2-8°C**.**

SYSTEM PARAMETERS

Wavelength	415 nm (Hg 405 nm)
Optical path	1 cm
Temperature	15-25°C
Zero Adjustment	Distalled water

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

A- Hemolysate preparation (Step 1):
1. Dispense 0.5 ml Lysing Reagent into tubes labeled as Control (C) and Test (T).
2. Add to it 0.1ml of the reconstituted control and well-mixed blood sample into the appropriately labeled tubes.
3. Mix until complete lysis is evident and allow to stand at room temperature for 5 minutes.

B- GHb separation (Step 2):
1. Remove cap from the Ion-Exchange Resin tubes, label as Control and Test.
2. Add 0.1 ml of the hemolysate from “Step 1” into the appropriately labeled Ion Exchange Resin tubes.

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 (Step 3):
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 1" into the appropriately
labeled tube.
3. Mix well and read each absorbance against distilled water

CALCULATION

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

$$\text{Ratio of Test (RT)} = \frac{\text{Abs. Test GHb}}{\text{Abs. Test THb}}$$

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

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**. Glycosylated hemoglobin reagent compared with commercial reagent of the same methodology performed on 20 human plasmas give a correlation of 0.979.

Measuring range
When run as recommended, BioScien Glycosylated hemoglobin reagent can measure concentration in the range of 4-15% in specimens.

Detection limit
The limit of detection is 3% HbA1c. The detection limit represents the lowest measurable HbA1c concentrations that can be distinguished from zero.

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

INTERFERING SUBSTANCES

Samples from patients with Hemoglobinopathies, decreased red cell survival times, gross lipemia may interfere with.

EXPECTED VALUES

Whole Blood	GHb%
Normal	< 8.0
Good Control	8.0 – 9.0
Fair Control	9.0 – 10.0
Poor Control	> 10.0

DYNAMIC RANGE

< 8.0%; It is recommended that each laboratory should establish its own reference range

LIMITATIONS OF PROCEDURE

1. Blood samples with Hemoglobin greater than 18g/dl should be diluted 1+1 with Normal saline before the assay.
2. 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.

REFERENCES

1. Trivelli, L.A., Ranney, H.M. and Lai, H.T., New Eng. J. Med. *284,

353 (1971).

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

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

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

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

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% and Mean Blood Glucose is obtained.

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	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	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	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

GHbA1	HbA1c	MBG
8.8	6.64	135
8.9	6.73	138
9	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	7.4	160
9.8	7.48	163
9.9	7.56	166
10	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	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	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

GHbA1	HbA1c	MBG
12.6	9.83	241
12.7	9.91	244
12.8	9.99	247
12.9	10.08	250
13	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	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	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	12.68	-
16.1	12.76	-
16.2	12.84	-
16.3	12.93	-

GHbA1	HbA1c	MBG
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	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	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	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	16.03	-

In the test study done by Nathan D.M et.al they calculated the Mean Blood Glucose concentration from the Value HbA1c% measured with the equation: MBG in mg/dl=33.3 xHbA1c value-86.
These values are linear in the range of 6.5-13%of HbA1c Values.