GLYCOSYLATED HEMOGLOBIN

INTENDED USE

For the quantitative determination of glycohemoglobin in blood.

METHOD

Ion Exchange Resin method.

CLINICAL SIGNIFICANCE

Glycosylated Hemoglobin (GHb) is formed continuously by the adduction of glucose by covalent 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 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.

REAGENTS

Ion Exchange Resin (Pre-dispensed Tubes)
Lysing Reagent
Control (10 %)
Resin Separators

REAGENT STORAGE 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.
- Reconstitute the control with 1 ml of distilled water. Allow to stand for 10 minutes 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.

PROCEDURE

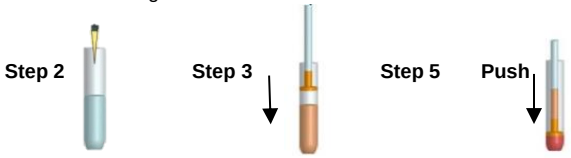
Wavelength	:	415 nm (Hg 405 nm)
Temperature	:	R .T.
Light path	:	1 cm

A. Hemolysate Preparation:

1. Dispense 0.5 ml Lysing Reagent into tubes labelled 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 until 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

- **With Control:**

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

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

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

- **Without control:**

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

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.

PERFORMANCE

Precision

The Intra assay precision was obtained by assaying three blood samples in replicates of 20 on the same day. Inter- assay precision was obtained by performing two runs per day of each of these same materials in duplicate over a span of 20 days. Results were as follows:

a- Intra assay precision

Sample	N	Mean (%)	SD (%)	% CV
Sample #1	20	5.7	0.18	3.2
Sample #2	20	7.7	0.19	2.4
Sample #3	20	13.1	0.24	1.8

b- Inter assay precision

Sample	N	Mean (%)	SD (%)	% CV
Sample #1	40	5.5	0.22	3.9
Sample #2	40	7.5	0.23	3.1
Sample #3	40	12.9	0.31	2.4

Linearity

The glycohemoglobin assay shows linearity for glycohemoglobin level in the range of 4.0 - 20.0%. Blood samples with total hemoglobin greater than 18 g/dl should be diluted x 2 with deionized water before assay.

Sensitivity

The sensitivity method is, in terms of detection limit (LOD), 4%.

Correlation

A comparative study of the present glycohemoglobin procedure and HPLC method gave the following results:
y=0.97x+2.34%, r=0.99.

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com

EC	REP	**Medical Device Safety Services** MDSS GmbH, Burckhardtstr. 1 30163 Hannover, Germany.

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 form. In such cases the incubation time during hemolysate preparation may be increased to 15 minutes to ensure elimination of this instable fraction.

BIBLIOGRAPHY

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).

ORDERING INFORMATION

REF	SIZE
11601	1 X 10 tests
11602	1 X 25 test
11603	1 x 25 tests + Control

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

GHbA1	HbA1c	MBG
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	---

GHbA1	HbA1c	MBG
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	---

GHbA1	HbA1c	MBG
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

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

Article # :116 -EN
Date of Revision : 03/2021

ISO 13485:2016 CE

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com

EC REP **Medical Device Safety Services**
MDSS GmbH, Burckhardtstr. 1
30163 Hannover, Germany.