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GLYCO HEMOGLOBIN

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

CLINICAL SIGNIFICANCE

Glyco Hemoglobin (GHb) is formed continuously by the adduction of glucose by covalent bonding to the terminal valine of the Hemoglobin beta chain progressively and irreversibly over a period of time and is stable till the life of the RBC. This process is 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 reliable indicator of the efficacy of therapy.

METHODOLOGY : Ion Exchange Resin Method

PRINCIPLE

Glyco Hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobin s HbA1 (HbA1a, A1b, A1c) which elute first during column chromatography. The Non glycosylated hemoglobin, which consists of the bulk of Hemoglobin has been designated HbA0. A hemolysed preparation 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, HbA0 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 Glyco hemoglobin is determined by measuring the ratio of the absorbances of the Glyco hemoglobin(GHb) and the Total hemoglobin(THb) fraction of the control and the sample.

STORAGE AND STABILITY

Reagents are stable at 2-8°C till the expiry mentioned on the labels.

Do not freeze.

The Resin separators can be removed on opening the kit and stored at R.T (below 30°C).

SPECIMEN

Venous blood is collected with EDTA/Heparin using aseptic techniques. GHb in blood is found to be stable for one week at 2-8°C.

ASSAY PROCEDURE

STEP A – Hemolysate Preparation

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

STEP B – Glyco hemoglobin (GHb) Separation

1. Remove cap from the Ion-Exchange Resin tubes and label as Control & Test.
2. Add 0.1 ml of the hemolysate form 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 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 curvette and measure each absorbance against distilled water.
7. Read the absorbances of the supernatant against distilled water at 415nm (405-420nm).

STEP C – Total Hemoglobin (THb) fraction

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

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Glyco Hemoglobin (Ion Exchange Resin Method)
Conversion table of GHB A_1 and A_{IC} to M.B.G
(Mean Blood Glucose)

A_1	A_{IC}	M.B.G	A_1	A_{IC}	M.B.G	A_1	A_{IC}	M.B.G
5.1	3.78	33	10.1	7.48	186	15.1	11.18	408
5.2	3.85	34	10.2	7.55	189	15.2	11.26	414
5.3	3.92	36	10.3	7.63	193	15.3	11.33	420
5.4	4.00	38	10.4	7.70	197	15.4	11.41	427
5.5	4.07	39	10.5	7.78	200	15.5	11.48	434
5.6	4.15	41	10.6	7.85	204	15.6	11.55	440
5.7	4.22	43	10.7	7.92	207	15.7	11.63	446
5.8	4.29	44	10.8	8.00	211	15.8	11.70	453
5.9	4.40	46	10.9	8.07	215	15.9	11.78	460
6.0	4.44	48	11.0	8.15	219	16.0	11.85	466
6.1	4.48	50	11.1	8.22	222	16.1	11.92	473
6.2	4.59	52	11.2	8.30	226	16.2	12.00	480
6.3	4.70	54	11.3	8.37	230	16.3	12.07	487
6.4	4.74	56	11.4	8.44	233	16.4	12.15	494
6.5	4.81	58	11.5	8.52	237	16.5	12.22	501
6.6	4.89	59	11.6	8.59	241	16.6	12.30	508
6.7	4.96	61	11.7	8.67	244	16.7	12.37	515
6.8	5.04	65	11.8	8.74	248	16.8	12.44	522
6.9	5.11	68	11.9	8.81	252	16.9	12.52	530
7.0	5.18	72	12.0	8.89	255	17.0	12.60	537
7.1	5.26	76	12.1	8.96	259	17.1	12.67	544
7.2	5.33	79	12.2	9.04	263	17.2	12.74	551
7.3	5.40	83	12.3	9.11	266	17.3	12.81	559
7.4	5.48	87	12.4	9.18	270	17.4	12.90	566
7.5	5.55	90	12.5	9.26	274	17.5	12.96	574
7.6	5.65	94	12.6	9.33	277	17.6	13.04	582
7.7	5.70	98	12.7	9.41	281	17.7	13.11	590
7.8	5.78	101	12.8	9.48	285	17.8	13.18	597
7.9'	5.85	105	12.9	9.55	288	17.9	13.26	605
8.0	5.92	109	13.0	9.63	292	18.0	13.33	613
8.1	6.00	112	13.1	9.70	295	18.1	13.41	621
8.2	6.07	116	13.2	9.78	299	18.2	13.48	629
8.3	6.15	120	13.3	9.85	304	18.3	13.55	637
8.4	6.22	123	13.4	9.92	309	18.4	13.63	645
8.5	6.29	127	13.5	10.00	314	18.5	13.70	653
8.6	6.37	131	13.6	10.10	320	18.6	13.78	662
8.7	6.44	134	13.7	10.15	326	18.7	13.85	670
8.8	6.52	138	13.8	10.22	331	18.8	13.92	678
8.9	6.59	142	13.9	10.30	337	18.9	14.00	686
9.0	6.67	145	14.0	10.37	342	19.0	14.07	694
9.1	6.74	149	14.1	10.44	348	19.1	14.15	704
9.2	6.81	152	14.2	10.52	353	19.2	14.22	712
9.3	6.89	156	14.3	10.59	359	19.3	14.30	720
9.4	6.96	160	14.4	10.67	365	19.4	14.37	729
9.5	7.04	164	14.5	10.74	371	19.5	14.44	738
9.6	7.11	167	14.6	10.81	377	19.6	14.52	746
9.7	7.18	171	14.7	10.89	383	19.7	14.59	755
9.8	7.26	175	14.8	10.96	389	19.8	14.67	764
9.9	7.33	178	14.9	11.04	395	19.9	14.74	773
10.0	7.41	182	15.0	11.11	401	20.0	14.81	782

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