



GLICOTEST HbA1c

Immunoturbidimetric test for the quantitative determination of Hemoglobin A1c (HbA1c) in human blood



ORDER INFORMATION

REF	Kit size
GD5424 00	1x30 + 1x10 + 1x125 ml
KL5424 00	1x30 + 1x9.5 + 1x125 ml
BK5424 00	1x(30+9.5+0.5 ml)

INDICATION

Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the addition of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period.

In a classical study, Trivelli et al showed Hemoglobin A1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serves as an indicator of metabolic control of the diabetic, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control.

Hemoglobin A1c has been defined operationally as the "fast fraction" hemoglobins (HbA1a, A1b, A1c) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA0. However, until the publication of the Diabetes Care & Complications Trial (DCCT) in 1993, the idea that better glycemic control yielded a better long-term prognosis was only a theory. The DCCT compared patients who had intensive therapy with patients who received conventional care for their Type 1 diabetes. The measurement of HbA1c was a prime factor in this study. It was found that the patients undergoing intensive therapy maintained lower mean blood glucose concentrations, indicated by their significantly lower HbA1c levels. These patients subsequently demonstrated significantly lower morbidity and mortality than the patients undergoing more conventional care. Their risk of retinopathy, nephropathy and neuropathy was reduced by approximately 40-75%. Thus, HbA1c levels were established as a critical indicator of long-term glycemic control in patients with Type 1 diabetes.^(2,3,4)

PRINCIPLE

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (RB), latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

COMPOSITION

REAGENT A (RA):

Latex	0.13%
Glycine buffer	20 mmol/l

REAGENT B1 (RB1):

Glycine buffer	80 mmol/l
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REAGENT B2 (RB2):

Anti-HbA1c (Mouse anti-human HbA1c monoclonal antibody)	0.05 mg/ml
Anti-IgG (Goat anti-mouse IgG polyclonal antibody)	0.08 mg/dl

REAGENT C (RC):

Hemolysis reagent

REAGENTS PREPARATION

RA and RC are supplied as ready to use liquids.

RB is prepared by pouring the entire contents of the RB2 vial into the RB1 vial. Mix gently. It is possible to prepare monoreagent RB by means of share of RB1 and RB2, keeping the dilution ratio (9.5 + 0.5).

When drawing RB1 and RB2 in share, it is compulsory to work with sterile disposable equipment. After drawing RB1 and RB2 in share store instantly the reagents at 2-8 °C.

When procedure described at point 3 is applied, it is compulsory to share the reagent RA too.

Incorrect application of the procedure of reagent preparation in share invalidates the quality of RB1 and RB2 reagents.

Storage and stability

All reagents are stable up to expiry date stated on the label, if stored at 2-8 °C.

Reagent RA is stable after opening for one month at 2-8 °C.

Monoreagent is stable (RB) one month at 2-8 °C.

REAGENT DETERIORATION

Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.

AUXILIARY REAGENTS

- 20 and 1000 µl pipettes
- 2 ml test tubes
- HbA1c Calibrator, REF GD5426 00
- HbA1c Controls, REF GD5428 00

SPECIMEN

Anti coagulated whole blood.

Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants are required. Collect venous blood with EDTA using aseptic technique. All human specimens should be regarded as potentially infectious.

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A37.⁽¹²⁾

Stability

Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8 °C.⁽⁵⁾

INTERNAL QUALITY CONTROL

The reliability of test results should be monitored whenever patient samples are assayed using a standard and quality control materials analyzed in the same manner employed for the unknowns. We suggest the use of commercially available Hemoglobin A1c controls with an assayed range. If controls do not fall into the assayed range patient values from that run should not be reported. The run should be repeated, making sure that all mixing and handling instructions are strictly followed.

Linearity of the assay should be verified with a commercial linearity check set, or dilutions of a high specimen, at least every six months.

PROCEDURE

Allow reagents to reach working temperature before using.

Hemolysate preparation

1. Dispense 1 ml hemolysis reagent (RC) into tubes labelled: Control, Patients, etc. Use plastic or glass tubes of appropriate size are acceptable.
2. Place 20 µl of well mixed whole blood into the appropriately labelled hemolysis reagent tube. Mix well the solution.
3. Allow to stand for 5 minutes or until complete lysis is evident.
4. Mix well again.

Hemolysate stability

Hemolysates may be stored up to 10 days at 2-8 °C.

Instruments

Refer to specific instrument application for suggested settings.

Procedure (Hitachi 717)

TEST NAME	HbA1c
ASSAY CODE	[1-POINT] : [50] - [0]
SAMPLE VOLUME	[6] [3]
R1 VOLUME	[210] [50][NO]
R2 VOLUME	[70] [20] [NO]
WAVELENGTH	[] [660]
CALIBRATION	[NONLINEAR] [4][5]
STD (1) CONC – POS	[0.0*] [1]
STD (2) CONC – POS	[**][2]
STD (3) CONC – POS	[**][3]
STD (4) CONC – POS	[**][4]
STD (5) CONC – POS	[**][5]
STD (6) CONC – POS	[**][5]
SD LIMIT	[999]
DUPLICATE LIMIT	[1000]
SENSITIVITY LIMIT	[0]
ABS LIMIT (INC/DEC)	[32000] [INCREASE]
PROZONE LIMIT	[-][-]
EXPECTED VALUE	[-][-]
PANIC VALUE	[-][-]
INSTRUMENT FACTOR	[1.0]

* Use saline solution for 0.0 calibrator

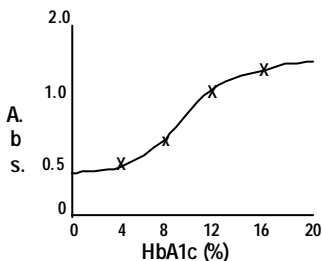
** Input the values of the calibrator set being used

Hitachi 717 is a registered trademark of Nissel Sangyo Co. Ltd, Japan.

CALCULATION OF RESULTS

HbA1c results for the unknowns and controls are determined using the prepared calibration curve.

Example of calibration curve (do not utilize to calculate the results)



Calibration curve is stable 15 days in most of instruments (utilizing calibrators REF GD5426 00, 4 levels).

EXPECTED VALUES⁽¹¹⁾

Non diabetic subjects	Diabetic subjects
< 6%	< 7%

Each laboratory should establish its own expected values. In using Hemoglobin A1c to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself.

There is a 3-4 weeks time lag before Hemoglobin A1c reflects changes in blood glucose level.

ANALYTICAL PERFORMANCES

Precision

The intra assay precision was established by assaying blood with three Hemoglobin A1c levels twenty times each. The inter assay precision was established by assaying the same three blood samples in duplicate for ten runs conducted over a five day period. The results are reported in the following tables:

a- Intra-assay

Level	Mean (%)	SD (%)	%CV
Low	4.76	0.08	1.26
Medium	7.29	0.08	1.10
High	10.09	0.16	1.47

b- Inter-assay

Level	Mean (%)	SD (%)	%CV
Low	4.72	0.06	1.27
Medium	7.36	0.08	1.09
High	11.00	0.17	1.55

Linearity

Assay range is 2.0%-16.0%.

Sensitivity

Sensitivity was investigated by reading the change in absorbance at 660nm for a saline sample and a whole blood sample with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used (Hitachi 717), the HbA1c reagent showed little or no drift on the zero sample. Under the reaction conditions described, a 0.073 absorbance change is approximately equivalent to 1.0% HbA1c.

Correlation

A study using 45 human specimens between this Hemoglobin A1c procedure and another automated immunoassay procedure (Roche Diagnostics) yielded the following results:

$$y = 1.05x - 0.36\% \quad r=0.995$$

Interferences

1. Serum components at the following concentrations do not interfere with this analytical method:

Bilirubin	50 mg/dl
Ascorbic Acid	50 mg/dl
Triglycerides	2000 mg/dl
Carbamylated hemoglobin	7.5 mmol/l
Acetylated hemoglobin	5.0 mmol/l

2. It has been reported that results may be inconsistent in patients who have the following condition: opiate addiction, lead poisoning, alcoholism, ingest large doses of aspirin.^(6,7,8,9)
3. It has been reported that elevated levels of HbF may lead to underestimation of HA1c and that uremia does not interfere with HbA1c determination by immunoassay.⁽¹⁰⁾

4. It has been reported that Hemoglobin variants HbS and HbA2 are not detected by immunoassay, leading to possible inaccurate determination. Also, it has been reported that labile intermediates (Schiff base) are not detected and do not interfere with HbA1c determination by immunoassay.⁽⁵⁾
5. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed

13. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

TEST LIMITS

1. This assay should not be used for the diagnosis of diabetes mellitus.
2. Patient specimens should always be assayed using a calibration curve.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium Azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended⁽¹³⁾.

Waste management

Please refer to local legal requirements.

INSTRUMENTS APPLICATION AVAILABLE FOR GLICOTEST HbA1c

- ABBOT AEROSSET
- ALYCON
- ATAC 8000
- BECKMAN CX
- COBAS MIRA NEW
- COBAS MIRA PLUS
- DIMENSION
- EXPRESS
- HBA1c-RA 500-1000
- HITACHI 704
- HITACHI 717
- HITACHI 902
- HITACHI 911
- HITACHI 917
- NEW AU400
- NEW AU600
- OLYMPUS AU400
- ROCHE MODULAR
- SELECTRA

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