Cromatest

LINEAR

Glycated HbA1c CE

CONTENTS			
REF	3155105 3155110	Glycated HbA1c	25 Tests 100 Test
For *in vitro* diagnostic use only			

GLYCATED HbA1c

Chromatographic determination in tube with preweighted resin of Hemoglobin A1c in blood

INDICATION

Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the addiction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is nonenzymatic, 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 better 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.

PRINCIPLE

The present procedure utilizes a weak binding cation-exchange resin for the rapid separation of glycated hemoglobin A1c from all the other hemoglobins.
A hemolyzed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbA0 binds to the resin. HbA0 consist of all the other hemoglobins except A1c which remains in solution. After the mixing period, a filter is used to separate the supernatant containing the A1c from the resin. The percent glycohemoglobin is determined by measuring the absorbance at 415 nm of the A1c fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent of HbA1c.

COMPOSITION

R1 Resin: Cation-exchange resin 8 mg/ml, buffered at pH 6.9.
3155105 25 x 2 mL; 3155110 100 x 2 mL

R2 Lysing solution: Potassium cyanide 10 mM, surfactant.
3155105 1 x 12,5 mL; 3155110 4 x 12,5 mL. R: 26/27/28

STANDAR. CALIBRATOR: Liophylized, HbA1c 10%.

FILTER SEPARATORS:
3155105 25 filters; 3155110 100 filters

REAGENTS PREPARATION

- Reagent 1 and Reagent 2 are ready to use.
- Glycohemoglobin Standard (CAL): add 1 mL of deionized water, wait 30 minutes, mixing gently by inversion.

Storage and stability

All reagents are stable to expiration date stated on the labels. Resin and Lysing Reagent may be stored refrigerated (2-8 °C).
The reconstituted standard is stable for at least 30 days if frozen at –20 °C.
Alterations in the physical appearance of the reagents may be an indication of reagent instability.

ANCILLARY EQUIPMENT

- Semi automatic pipettes of 10, 200 and 1000 µL.
- Chronometer.
- Glass or plastic test tubes to hold 0.6 ml and 5 mL.
- Rocker or rotator.
- Spectrophotometer or colorimeter set at 415 nm.
- Glycohemoglobin quality control materials.

SPECIMEN

Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than the anticoagulants are required. Collect venous blood with EDTA using aseptic technique. Glycohemoglobin in whole blood collected with EDTA is stable for one week at 2 – 8 °C. Avoid using lipemic samples.
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3[7].

INTERNAL QUALITY CONTROL

The reliability of test results should be monitored routinely using stable quality control materials and analyzed in the same manner employed for the unknowns.

PROCEDURE

Allow reagents to reach working temperature before using.

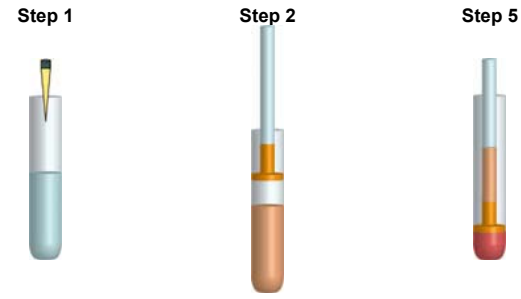
Hemolysate Preparation:

1. Dispense 500 µL Lysing Reagent (Reagent 2) into tubes labeled: Standard, Control, Sample 1, etc.
2. Place 100 µL of the well-mixed blood sample, standard or control into the appropriately labeled tube. Mix well.
3. Allow to stand for 5 minutes.

Glycohemoglobin preparation:

1. Add 70 µL of the hemolysate in the resin tube (Reagent 1).
2. Position the Filter Separators in the tubes so that the rubber sleeve is approximately 1 cm above the liquid level.
3. Place the tubes on the rocker or rotator and mix continuously for 5 minutes.
4. Remove the tubes from the rocker or rotator.
5. Push the Filter Separator into the tubes until the resin is firmly packed.

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687



6. The supernatant may be poured into another tube or directly into a cuvette for absorbance measurement.
7. Adjust the instrument to zero absorbance at 415 nm with deionized water as the blank. (Wavelength range: 390-420).
8. Read and record the absorbance values for Standard, Control, Sample 1, etc. These readings are for glycohemoglobin.

Total Hemoglobin Fraction:

1. Dispense 5.0 mL deionized water into tubes labeled: Standard, Control, Sample 1, etc.
2. Place 20 µL of the hemolysate into the appropriately labeled tube. Mix.
3. Adjust the instrument to zero absorbance at 415 nm with deionized water as the blank.
4. Read and record the absorbance values for Standard, Control, Sample 1, etc. These readings are for total hemoglobin.

CALCULATION OF RESULTS

Results should be determined as follows:

$$\%\ HbA1c\ (unknown) = \frac{R\ (unknown)}{R\ (standard)} \times \text{standard conc}$$

where:

$$R\ (unknown) = \text{Ratio (unknown)} = \frac{\text{Abs of HbA1c (unknown)}}{\text{Abs of Hb Tot (unknown)}}$$

$$R\ (standard) = \text{Ratio (standard)} = \frac{\text{Abs of HbA1c (standard)}}{\text{Abs of Hb Tot (standard)}}$$

Example:
A standard containing 8.0% glycohemoglobin had Abs. = 0.480 for the HbA1c and Abs. = 0.575 for the Hb Tot. An unknown sample had HbA1c Abs. = 0.962 and Hb Tot Abs. = 0.746. The glycohemoglobin concentration of the unknown is calculated as follows:

$$R\ (unknown) = \frac{0.962}{0.746} = 1.289$$

$$R\ (standard) = \frac{0.480}{0.575} = 0.835$$

$$\%\ Glyco\ (unknown) = \frac{1.289}{0.835} \times 8.0 = 12.4$$

EXPECTED VALUES

6.0 – 8.6%. This range represents the 95% confidence interval for 100 outpatient subjects with normal glucose values and no history of diabetes. A study of 31 diabetic subjects showed glycohemoglobin values from 8.4% to 16.0%. For the diabetic population, a comparison of the fasting plasma glucose with the glycohemoglobin level gave a correlation coefficient equal to 0.84.
Each laboratory should establish reference ranges for its own patient population.

ANALYTICAL PERFORMANCES

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:

$y = 0.97x + 2.34\ \%,\ r = 0.99.$

Interferences

1. 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[6].
2. 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].
3. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

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[8].

Waste management

Please refer to local legal requirements.

REFERENCES

1. Trivelli, L.A., Ranney, P.H., New Eng. J. Med. 284, 353 (1971).
2. Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978).
3. Gabbay, K.H. Hasty, K., Breslow. J. L., Ellison, R.C. Bunn. H.F., and Gallop, P.M.J. Clin. Endocrinol. Metab, 44, 859 (1977).
4. Bates, H.M., Lab Manag., Vol. 16 (Jan. 1978).
5. Tietz, N,W., Textbook of Clinical Chemistry, Philadelphia, W.B. Saunders Company, p.794-795 (1999).
6. Engbaek, F., et al, Clin. Chem. 35, pp. 93-97 (1989).
7. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
8. 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.

B3155-1/0902
R1.ing

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687