COD 31047 1 x 60 mL
STORE AT 2-8ºC
Reagents for measurement of Hemoglobin A1C concentration Only for *in vitro* use in the clinical laboratory

HEMOGLOBIN A1C-DIRECT (HbA1C-DIR)

BioSystems REAGENTS & INSTRUMENTS

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DIRECT

PRINCIPLE OF THE METHOD

After preparing the hemolysate, the Hemoglobin A1C (HbA1C) concentration is quantified by a latex turbidimetric assay. The different hemoglobins present in the hemolysate are unspecifically adsorbed on the latex particles surface in a ratio equivalent to their concentration in the sample. The addition of an anti-human HbA1C antibody causes agglutination that is proportional to the concentration of hemoglobin A1C and can be measured by turbidimetry.

CONTENTS AND COMPOSITION

A. Reagent. 1 x 50 mL. Suspension of latex particles, sodium azide 0.95 g/L, pH 8.0.

B. Reagent. 1 x 10 mL. Anti-human HbA1C antibody, stabilizers, pH 6.0.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: absorbance of the blank over 0.700 at 670 nm.

AUXILIARY REAGENTS

S. HbA1C Direct Standards (Cod. 31048). 4 levels for 0.5 mL. Human blood. HbA1C concentration is given on the label.

Human blood used in the preparation of the standard has been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the standard should be handled cautiously as potentially infectious.

Reconstitute with 0.5 mL of distilled water. Stable for 30 days at 2-8°C.

REAGENT PREPARATION

Reagents are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC.
- Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 670 nm.

SAMPLES

Venous blood collected by standard procedures and with EDTA as anticoagulant.

HbA1C in blood is stable 7 days at 2-8ºC.

REFERENCE VALUES

The following cut-off points have been established by the Diabetes Control and Complications Trial Research Group (DCCT) and have been adopted by many countries for a reference population (Non diabetic) and for the evaluation of the degree blood glucose control in diabetic patients[1,2].

IFCC (mmol/mol)	NGSP-DCCT (%)	Reference values/Degree of control
20 - 48	4.0 - 6.5	Non Diabetic
42 - 53	6.0 - 7.0	Goal
53 - 64	7.0 - 8.0	Good Control
> 64	> 8.0	Action suggested

CALIBRATION

Calibration curve: Calculate the absorbance difference ($\Delta A_{Standard} - \Delta A_{Blank}$) of each point of the calibration curve and plot the values found against the HbA1C concentration. HbA1C concentration in the sample is calculated by interpolation of its absorbance difference ($\Delta A_{Sample} - \Delta A_{Blank}$) on the calibration curve.

It is recommended to do a reagent blank every day and a calibration at least every 30 days, after reagent lot change or as required by quality control procedures.

PROCEDURE

Hemolysate preparation

The calibrators should be treated as patient samples.

1. Pipette into a test tube:

Blood	50 µL
Distilled water	5.0 mL

2. Mix. Incubate at room temperature for 5 minutes.

The hemolysate is stable 72 hours at 2-8ºC.

Assay (Note 1)

1. Bring the Reagents and the instrument to 37°C.
2. Pipette into a cuvette:

	Macro	Micro
Reagent A	950 µL	450 µL
Blank (Dist. Water), Standard or Sample	15 µL	7 µL

3. Mix and insert the cuvette into the instrument. Start stopwatch and after 2 minutes:
4. Pipette into the cuvette:

Reagent B	200 µL	100 µL

5. Mix and read the absorbance at 670 nm after 10 seconds (A1) and after 5 minutes (A2).
6. Calculate the absorbance difference $\Delta A = A_2 - A_1$.

CALCULATION

The HbA1C values obtained are traceable to IFCC Reference Method.

The traceable values to Reference Method as described by the US National Glycohemoglobin Standardization Program (NGSP) are calculated using the following general formula[3].

%HbA1C-NGSP-DCCT (%) = 0.0915 x HbA1C-IFCC (mmol/mol) + 2.15

QUALITY CONTROL

It is recommended to use the Hemoglobin A_{1c} Controls, Normal (cod. 18001) and Elevated (cod. 18002), to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

The metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

- Detection limit: 6 mmol/mol.
- Measurement interval: (approximate value dependent on the highest standard concentration): 6 - 140 mmol/mol. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Mean concentration	CV	n
37 mmol/mol	1.8 %	15
78 mmol/mol	1.6 %	15

- Reproducibility (run to run):

Mean concentration	CV	n
37 mmol/mol	3.1 %	15
78 mmol/mol	3.0 %	15

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 4g/L) and bilirubin (10 mg/dL) do not interfere. Other drugs and substances may interfere[4].

  In the immunoassay methods, the presence of acetylated-Hb, carbamylated-Hb, labile HbA1C HbE and HbD do not affect the results[5,6]. Other hemoglobin variants like HbS, HbF or HbC can interfere [5].

  In hemolytic anemia, iron deficiency anemia and transfusion, the average age of erythrocytes is altered. Caution should be used when interpreting the HbA1C results from patients with these conditions.

DIAGNOSTIC CHARACTERISTICS

HbA1C is the product of the irreversible condensation of glucose with the N-terminal residue of the β-chain of hemoglobin A.

The HbA1C concentration in blood is directly proportional to the mean concentration of glucose prevailing in the previous 6-8 weeks, equivalent to the lifetime of the erythrocytes[1], and the estimated average glucose (eAG) during this period can be calculated with the formulas below[7].

eAG (mg/dL) = 28.7 x HbA1C-NGSP-DCCT (%) – 46.7

eAG (mmol/L) = 1.59 x HbA1C-NGSP-DCCT (%) – 2.59

eAG (mg/dL) = 2.64 x HbA1C-IFCC (mmol/mol) + 15.0

eAG (mmol/L) = 0.146 x HbA1C-IFCC (mmol/mol) + 0.843

HbA1C levels are a valuable adjunct to glucose determinations in the assessment and follow up of individuals with diabetes mellitus, providing much more reliable information for glycemia monitoring than do determinations of glucose. Numerous studies have shown that diabetes related complications may be reduced by the long term monitoring and tight control of blood glucose levels. The HbA1C concentration may also be a useful tool in the diagnosis of diabetes[8].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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8. Nathan DM, et al. International Expert Committee report on the role of the HbA1C assay in the diagnosis of diabetes. Diabetes care 2009: 32: 1327-1334.