HEMOGLOBIN A1C-DIRECT (HbA1C-DIR)

BioSystems

COD 13047 1 x 60 mL

For *in vitro* use in the clinical laboratory only

HEMOGLOBIN A1C-DIRECT (HbA1C-DIR)
DIRECT

INTENDED USE

Reagents for the measurement of hemoglobin A1C (HbA1C) concentration in human blood. The obtained values are useful as an aid in the diagnosis and monitoring of diabetes mellitus.

These reagents are for use in the BioSystems A25 and A15 analyzer or in other analyzer with similar performance characteristics. The reagents may also be used by a manual procedure.

CLINICAL SIGNIFICANCE

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[2].

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 glycaemia 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[3].

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

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 AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 30 days.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

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.

SAMPLES

Venous blood collected by standard procedures and with EDTA as anticoagulant.
HbA1C in blood is stable 7 days at 2-8ºC.

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 gently. Avoid the formation of foam. Incubate at room temperature for 5 minutes.

The hemolysate is stable 72 hours at 2-8ºC.
Test according to Test Parameters.

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[4].

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

CALIBRATION

A reagent blank should be done every day and a calibration at least every 30 days, after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the Hemoglobin A1C Controls, Normal (cod. 18001) and Elevated (cod. 18002) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

REFERENCE VALUES

The following cut-off points have been established and adopted by many countries for a reference population (Non diabetic) and for the evaluation of the degree blood glucose control in diabetic patients[1,5].

IFCC mmol/mol)	NGSP-DCCT (%)	Reference values/Degree of control
20 - 38	4.0 - 5.6	Non Diabetic
39 - 47	5.7 - 6.4	Pre-diabetic
> 48	> 6.5	Diabetic
< 53	< 7.0	Treatment Goal

This range is given for orientation only; each laboratory should establish its own reference range.

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer. Results are similar with A15.

- Detection limit: 6 mmol/mol.
- Measurement interval: (approximate value dependent on the highest standard concentration): 6 - 140 mmol/mol.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
37 mmol/mol	1.8 %	3.1 %
78 mmol/mol	1.6 %	3.0 %

- 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.

LIMITATIONS OF THE PROCEDURE

- Interferences: Bilirubin (up to 10 mg/dL) and Lipemia (triglycerides up to 400 mg/dL) do not interfere. Other drugs and substances may interfere[6].
  In the immunoassay methods, the presence of acetylated-Hb, carbamylated-Hb, labile HbA1C HbE and HbD do not affect the results[7,8]. Other hemoglobin variants like HbS, HbF or HbC can interfere[7].
  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.

NOTES

1. To avoid possible interferences by other assays performing HbA1C determinations in different runs of the other test is recommended.

BIBLIOGRAPHY

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8. Little R.R., Rohlfing C; Hanson S, Connolly S, Higgings T, Weykamp C, D'Costa M, Luzzi V, Owen W, Roberts WL. Effects of Hemoglobin (Hb) E and HbD traits on measurements of Glycated Hb (HbA1C) by 23 methods. Clin Chem 2008, 54: 1277-1282.

TEST PARAMETERS (Notes 1 & 2)

These reagents may be used in several automatic analyzers. Specific instructions for application in many of them are available on request.

R1: Use Reagent A, R2: Use Reagent B.

	A25	A15
GENERAL		
Name	**HbA1C-DIR**	**HbA1C-DIR**
Sample type	WBL	WBL
Analysis mode	fixed-time bir.	fixed-time bir.
Units	mmol/mol	mmol/mol
Turbidimetry test	yes	yes
Decimals	0	0
Type of reaction	increasing	increasing
PROCEDURE		
Reading mode	monochromatic	monochromatic
Main filter	670	670
Reference filter	-	-
Sample	3	3
Vol. R1	190	190
Vol. R2	40	40
Washing	1.2	1.2
Reading 1 (cycle)	10	7
Reading 2 (cycle)	30	20
Reagent 2 (cycle)	9	6
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	specific	specific
Number of calibrators	4	4
Calibration curve	increasing poligonal	increasing poligonal
OPTIONS		
Blank absorbance limit	0.700	0.700
Kinetic blank limit	-	-
Linearity limit	-	-
Substrate depletion	-	-

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