CE IVD DIALAB

Liquid Reagents – ready to use

HEMOGLOBIN A_{1C}

Ion Exchange
3 Reagents + Columns

Diagnostic reagent for quantitative in vitro determination of hemoglobin A_{1C} (HbA_{1C}) in human whole blood on photometric systems

REF Cont.

601100	**40 tests**	1 x 9.6 ml	Reagent 1
		1 x 96 ml	Reagent 2
		1 x 680 ml	Reagent 3
		1 x 40 pcs	Microcolumns

Additionally offered:

605803	3 x 1 ml	HbA1c Control Set (3 levels)

TEST PARAMETERS

Method:	Colorimetric, Ion exchange column
Wavelength	415 nm, (405-425 nm)
Temperature:	21 - 26°C
Sample:	whole blood
	Heparin or EDTA may be used as anticoagulants.
Linearity:	at least 17.0%
Sensitivity:	lower than 4.3%

REAGENT COMPOSITION

COMPONENTS	FINAL CONCENTRATIONS
Reagent 1	
Potassium phtalate pH 5,0	50 mmol/L
Reagent 2	
Phosphate Buffer pH 6,5	30 mmol/L
Reagent 3	
Phosphate Buffer pH 6,5	72 mmol/L
Microcolumns	
Resin equilibr. in PPS pH 6,5	72 mmol/L

REAGENT PREPARATION

Reagents are ready to use.
The long-term storage of the columns leads to an excessive packing of the resin diminishing the flow rate and lenghtening the elution step. To regain the flow efficiency it is advisable 10 minutes before starting the test, to invert the columns to resuspend the contents, place them back to their upright position and let the resin settle for a few minutes.
Some air bubbles may occasionally appear inside the resin bed. Their presence do not alter the test performance.

REAGENT STABILITY AND STORAGE

Conditions:	close immediately after use avoid contamination
Storage temperature:	15 – 30°C
Stability:	up to the expiration date

SAMPLE STABILITY AND STORAGE

Stability: at 2 – 8°C 10 days
Discard contaminated specimens.

INTERFERING SUBSTANCES

no interference up to
bilirubin 20 mg/dl
triglyceride 1000 mg/dl
Some drugs and other substances may interfere.
In the ionic exchange chromatographic methods, the presence of hemoglobin C or S in the sample may slightly alter results, but differences are not clinically significant[5]. Other hemoglobin variants like HbE, HbF, carbamyl-Hb and acetyl-Hb can interfere[5,6]. The incubation with Reagent 1 eliminates the interference due to HbA1c-labile.
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.

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature (21-26°C)
Use only microcolums and reagents of the same lot number.

Hemolysate preparation and labile fraction elimination

Pipette into test tubes	
Blood	50 µl
Reagent 1	200 µl
Shake thoroughly and let it stand at room temperature for 10-15 min. Then prepare the column	

Column preparation

- Before placing the column into a tube, keep it standing upside down for 10 min. to improve the fluidity.
- Remove the upper cap of the column.
- Push the upper filter disc down to the surface of the resin by using the flat end of a pipette. Take care not to compress the resin.
- Then snap the tip off the bottom.
- Let the column drain completely to waste.

Separation and reading of HbA_{1C} fraction

Pipette on the upper filter	
Hemolysate	50 µl
Let the column drain to waste	
In order to drain any sample residue left above the upper disc pipette after 1 minute:	
Reagent 2	200 µl
Let the column drain to waste and pipette:	
Reagent 2	2000 µl
Let the column drain to waste. Then place the column over a new test tube and add:	
Reagent 3	4000 µl
Collect the eluate (=HbA_{1C} fraction), shake thoroughly and read the absorbance A (HbA_{1c}) of the HbA_{1c} fraction at 415 nm against dist. water. The absorbance is stable for at least one hour.	

Reading of Hb $_{TOTAL}$

Pipette into a test tube	
Reagent 3	12000 µl
Hemolysate	50 µl
Shake thoroughly and read the absorb. A (Hb_{TOTAL}) of the Hb_{TOTAL} fraction at 415 nm against dist. water. The absorbance is stable for at least one hour.	

CALCULATION (light path 1 cm)

HbA1C percentage in the sample:

$$HbA_{1C}\ (\%) = \frac{A\ (HbA_{1C})}{A\ (Hb_{TOTAL})} \times \frac{100}{3}$$

The results obtained with the present method can be converted into equivalent to a US National Glycohemoglobin Standardization Program certified method (NGSP) or equivalent to the International Federation of Clinical Chemistry standardized method (IFCC), using the following formulas:

HbA1c-NGSP [%] = 0.86 HbA1c-Dialab [%] + 0.24
HbA1c-IFCC [mmol/mol]* = 9.4 HbA1c-Dialab [%] – 20.9
*new IFCC units

TEMPERATURE CORRECTION FACTORS

The test is designed for a working temperature of 21 –26°C. For other temperatures multiply results by the corresponding correction factor below:

Factor for 18 – 20°C	1.15
Factor for 27 – 30°C	0.90

REFERENCE RANGE

The following cut-off points have been established by the Diabetes Control and Complications Trial Research Group 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[2,3].

Degree of Control	DCCT/NGSP [%]	IFCC [mmol/mol]	Dialab [%]
non diabetic	4.0 – 6.0 %	20 – 42	4.4 – 6.7
Goal	6.0 – 6.5 %	42 – 48	6.7 – 7.3
Good control	6.5 – 8.0 %	48 – 64	7.3 – 9.1
Action suggested	> 8.0 %	> 64	> 9.1

* It is recommended that each laboratory establishes its own normal range.

DIAGNOSTIC CHARACTERISTCS

HbA_{1C} is the product of the irreversible condensation of glucose with the N-terminal Amino acid residue of the β-chain of hemoglobin A.
The HbA1C concentration in blood is directly proportional to the mean concentration of glucose in blood (MBG) as stated in the formulas below, for an extended period of time (6-8 weeks)[2].

MBG (mg/dl) = 31.7 x %HbA1C – 66.1

MBG (mmol/l) = 1.76 x %HbA1C – 3.67

HbA1C levels are a valuable adjunct to blood glucose determination in the assessment of glycemic control for monitoring individuals with diabetes mellitus. However, it is not reliable for the diagnosis of diabetes[2,3].

TEST PRINCIPLE

After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin.
Hemoglobin A_{1C} is specifically eluted after washing away the HbA_{1a+b} fraction, and is quantified by direct photometric reading at 415nm.

PERFORMANCE CHARACTERISITICS

PRECISION (at 37°C)

Intra-assay n = 20	Mean Conc. [%]	CV [%]
Sample 1	7.2	5.4
Sample 2	9.9	6.3

Inter-assay n = 25	Mean Conc. [%]	CV [%]
Sample 1	7.2	7.3
Sample 2	9.9	5.9

METHOD COMPARISON

When compared with an NGSP certified method (x), the following correlation was obtained:

y = 1.17 x – 0.28

QUALITY CONTROL

All control sera with HbA1C values determined by this method can be used.
We recommend:

REF Cont.

605803 3 x 1 ml HbA1c Control Set (3 levels)

WARNINGS AND PRECAUTIONS

Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

1. Bissé E, Abraham EC. New less temperature-sensitive microchromatographic method for the separation and quantitation of glycosylated hemoglobins using a non-cyanide buffer system. *J. Chromatog*. 1985; 344; 81-91.
2. Tietz NW. Clinical guide to laboratory tests, 3rd ed. Saunders Co., 1999.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329; 977 – 986.
4. Young DS. Effects of drugs on clinical laboratory tests, 4th ed. AACC Press, 1995.
5. Roberts WL et al. Effects of hemoglobin C and S traits on eight glycohemoglobin methods. *Clin Chem* 2002; 48: 383-385
6. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem* 2001; 47: 153-163

15°C 30°C IVD

DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A – 2351 Wiener Neudorf, Austria
IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0
Fax: ++43 (0) 2236 660910-30 e-mail: office@dialab.at