CORMAY HbA_{1C} DIRECT

DIAGNOSTIC KIT FOR DETERMINATION OF HAEMOGLOBIN A_{1C} CONCENTRATION

II GENERATION

Kit name	Cat. No
CORMAY HbA1C DIRECT 500	1-310
CORMAY HbA1C DIRECT "bulk"	1-257

INTRODUCTION

The determination of HbA_{1C} is most commonly performed for the evaluation of glycemic control in diabetes mellitus. HbA_{1C} values provide an indication of glucose levels over the preceding 4-8 weeks. Throughout the circulatory life of the red cell, hemoglobin A_{1C} is formed continuously by the adduction 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 [1] showed hemoglobin A_{1C} in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that hemoglobin A_{1C} serve as an indicator of metabolic control of the diabetic, since hemoglobin A_{1C} levels approach normal values for diabetics in metabolic control [2,3,4].
Hemoglobin A_{1c} has been defined operationally as the "fast fraction" hemoglobins (HbA_{1a}, A_{1b}, A_{1c}) 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 HbA_0.

METHOD PRINCIPLE

Method for hemoglobin A_{1C} determination complies with standardized method certified by the National Glycohemoglobin Standardization Program (NGSP).
The present method utilizes the interaction of antigen and antibody to directly determine the HbA_{1C} concentration in whole blood.
Total hemoglobin and HbA_{1C} have the same unspecific absorption rate to latex particles. When mouse antihuman HbA_{1C} monoclonal antibody is added , latex-HbA_{1C}-mouse anti human HbA_{1C} 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 HbA_{1C} absorbed on to the surface of latex particles.
The amount of agglutination is measured as absorbance.
The HbA_{1C} value is obtained from a calibration curve.

REAGENTS

Package

	CORMAY HbA1C DIRECT 500	CORMAY HbA1C DIRECT "bulk"
REAGENT 1	2 x 375 ml	--*
REAGENT 2	1 x 250 ml	--*
HEMOLYSING REAGENT	2 x 1000 ml	--*

* reagent volume is printed on the label.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. On board stability of the reagents depends on type of analyser used for analysis. Protect from light and avoid contamination!.

Concentrations in the test

latex	0.13%
mouse anti-human HbA_{1C} monoclonal antibody	0.05 mg/ml
goat anti-mouse IgG polyclonal antibody	0.08 mg/dl
stabilizers	
buffer	

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the intended purpose by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin [6, 7, 8, 9].
- It has been reported that elevated levels of HbF may lead to underestimation of HA_{1C} and, that uremia does not interfere with HbA_{1C} determination by immunoassay [10].
- This assay should not be used for the diagnosis of diabetes mellitus, but for monitor diabetic patients.
- In using Hemoglobin A_{1C} to monitor diabetic patients, results should be interpreted individually.
- Reagent HEMOLYSING REAGENT (Cat. No 4-398) can be ordered separately.
- Any clinical case with shortened erythrocyte survival (eg. hemolytic anemia, blood loss, pregnancy) might cause decrease in HbA1c values.

ADDITIONAL EQUIPMENT

- automated clinical chemistry analyser capable of accommodating two-reagent assays;
- general laboratory equipment;

SPECIMEN

Venous blood collected with EDTA.
Hemoglobin A_{1C} in whole blood collected with EDTA is stable for 7 days at 2-8°C.
Nevertheless it is recommended to perform the assay with freshly collected samples!

Sample pretreatment:

1. Dispense 500 µl HEMOLYSING REAGENT into tubes labeled: Control, Patients, etc.
2. Place 10 µl of well mixed whole blood into the appropriately labeled lyse reagent tube. Mix well and allow to stand for minimum 5 minutes, until complete lysis is evident. Next mix sample for 5 minutes.
3. The treated sample may be stored up to 10 days at 2-8°C. Mix sample again for 5 minutes before measurement.
4. **Note:** calibrators and controls should be also hemolysed according to sample pretreatment.

PROCEDURE

wavelength	660 nm (630-670 nm)
temperature	37°C

These reagents may be used in automatic analysers according to their service manual. Applications for analysers are available on request.

Test result is read automatically and the value is reported in % of heamoglobin unit in accordance with NGSP standardization.
In order to convert the result reported in % haemoglobin (NGSP) to value reported in SI units mmol/mol in accordance with IFCC standardization, the following master equation should be used:

$$HbA1c\ [mmol/mol\ IFCC] = (HbA1c\ [\%\ NGSP] - 2.15) \times 10.929$$

REFERENCE VALUES [11]

Non-diabetes < 6%
Patients with diabetes, control of glycaemia < 7%
It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY HbA_{1C} DIRECT CONTROLS (Cat. No 4-328) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY HbA_{1C} DIRECT CALIBRATORS (Cat. No 4-308) are recommended.

Controls and calibrators should be treated with HEMOLYSING REAGENT.

Calibration stability depends on type of analyser used for analysis. The calibration curve should be prepared with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using automatic analysers Biolis 24i Premium and Hitachi 717. Results may vary if a different instrument is used.

- **Analytical range:** 2 – 16% (up to 151 mmol/mol).

- **Specificity / Interferences**

Bilirubin up to 50 mg/dl, triglycerides up to 2000 mg/dl, ascorbate up to 50 mg/dl, carbamylated Hb up to 7.5 mmol/l and acetylated Hb up to 5.0 mmol/l do not interfere with the test.

- **Precision (% HbA_{1C})**

Repeatability (run to run) n = 10	Mean [%]	SD	CV [%]
level 1	6.06	0.06	0.99
level 2	11.30	0.07	0.65

Reproducibility (day to day) n = 20	Mean [%]	SD	CV [%]
level 1	5.95	0.190	3.19
level 2	8.34	0.093	1.12
level 3	12.15	0.179	1.47

- **Method comparison**

A comparison between HbA1C values determined at Biolis 24i Premium (y) and at ADVIA 1650 (x) using 80 samples gave following results:

$y = 0.890 x + 0.746$

$R = 0.9803$ (R - correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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