

CORMAY HbA_{1C}

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



Kit name	Kit size	Cat. No
CORMAY HbA _{1C}	2 x 62.4+2 x 40.6+2 x 50	4-593

INTRODUCTION

Haemoglobin A_{1C} (HbA_{1C}) is a reaction product of glucose and the N-terminal group of beta chains.

The level of HbA_{1C} is proportional to the level of glucose in blood and has been widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 6-8 weeks.

The measurement of HbA_{1C} is used in the long-term monitoring of diabetes mellitus.

METHOD PRINCIPLE

Both the concentration of HbA_{1C} and the concentration of total haemoglobin are measured in the test. The reported HbA_{1C} result is calculated as a % of total haemoglobin concentration.

After lysis of red blood cells the haemoglobin chain is hydrolysed by a protease and haemoglobin derivatives are converted to alkaline haematin, which is measured by absorbance changing at $\lambda=600$ nm. HbA_{1C} is measured using a latex agglutination inhibition rate assay. HbA_{1C} (in the sample) competes with the agglutination reagent (2-REAGENT HbA_{1C}) for antibody sites. The increase in absorbance ($\lambda=700$ nm) is inversely proportional to the concentration of HbA_{1C} in the sample. Concentration of HbA_{1C} is determined by interpolation from a calibration curve

REAGENTS

Package

Hb-DENATURANT	2 x 62.4 ml
Hb-REAGENT	2 x 40.6 ml
1-REAGENT HbA _{1C}	1 x 50 ml
2-REAGENT HbA _{1C}	1 x 50 ml

Reagent preparation and stability

The reagents are ready to use.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. Protect from light, don't freeze.

The reagents are stable for 30 days on board the analyser at approximately 10°C.

Concentrations in the test

sodium hydroxide	0.4% w/v
triton	2.5% w/v
octylphenyloxyethoxyethanol	2.5% w/v
HbA _{1C} antibody (mouse) coupled particles	0.1% w/v
non ionic surfactant	0.6% w/v
proclin 150	0.1% w/v

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Do not pipette by mouth.
- Reagent should be mixed thoroughly and equilibrated to system temperature for approximately ½ hour prior to use on the system.
- Hb-REAGENT contains sodium azide. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

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

SPECIMEN

Venipuncture in capillary blood samples may be used. Potassium EDTA or ammonium heparin are recommended as anticoagulants. Potassium EDTA or ammonium heparin whole blood is stable at -70°C for 6 months, at 5°C for 2 weeks, or up to 25°C for 1 week. Frozen samples should be thawed at room temperature, mixed thoroughly prior to use and should be not be refrozen.

PROCEDURE

These reagents may be used in automatic analysers according to their service manual. These reagents may be used directly in HITACHI 911/912 analysers. Applications for analysers are available on request.

Sample pretreatment:

Mix 10 µl of the whole blood sample with 400 µl of Hb-DENATURANT. Avoid foaming. Incubate for a minimum of 5 minutes at room temperature.

The treated sample may be stored up to 8 hours at room temperature or up to 48 hours at 2-8°C, if stored in a sealed container.

Total haemoglobin assay (T.Hb)

Pretreatment sample and Hb-REAGENT should be used for assay.

The assay prepare according to applications for analyser.

HbA_{1C} assay

Pretreatment sample, 1-REAGENT HbA_{1C} and 2-REAGENT HbA_{1C} should be used for assay.

The assay prepare according to applications for analyser.

Calculation

$$\%HbA_{1C} = \frac{HbA_{1C} \text{ (g/dl)}}{T. Hb \text{ (g/dl)}} \times 100$$

REFERENCE VALUES

nondiabetics	4-6 % HbA _{1C}
controlled diabetics	6-8% HbA _{1C}
uncontrolled diabetics	> 20 % HbA _{1C}

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it are recommended to use the CORMAY HbA_{1C} CONTROLS (Cat. No 4-319) with each batch of samples.

Controls should be treated with Hb-DENATURANT.

For the calibration of automatic analysers systems the CORMAY HbA_{1C} CALIBRATORS (Cat. No 4-318) are recommended:

- CALIBRATOR 1 for the determination of total haemoglobin.
- CALIBRATOR 1-6 for the determination of HbA_{1C}.

Calibrators shouldn't be treated with Hb-DENATURANT.

A multi point calibration is recommendet:

- every 30 days,
- with change of reagent lot/bottle,
- as indicated by quality control procedures.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using an automatic analyser HITACHI. Results may vary if a different instrument is used.

- Sensitivity:** 0.3 g/dl (HbA_{1C}).
- Linearity:** up to 23 g/dl (total haemoglobin).

- **Analytical range:** 7 g/dl – 23 g/dl (total haemoglobin),
0,3 g/dl – 2.06 g/dl (HbA_{1C}).
Samples with values above 14.7% (2.06 g/dl HbA_{1C} at a total haemoglobin of 14 g/dl) should not be diluted and result should be reported as >14.7% HbA_{1C}.

- **Specificity / Interferences**

Bilirubin up to 30 mg/dl triglycerides up to 1600 mg/dl, RF up to 2000 U/l, acetylsalicylic acid up to 60 mg/dl, ascorbate up to 30 mg/dl, sodium cyanate up to 50 mg/dl, urea up to 500 mg/dl do not interfere with the test.

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

Repeatability (within run) n = 20	Mean [%]	SD	CV [%]
level 1	5.337	0.163	3.06
level 2	7.927	0.238	3.00
level 3	10.518	0.251	2.38

Reproducibility (run to run) n = 20	Mean [%]	SD	CV [%]
level 1	5.663	0.194	3.43
level 2	8.316	0.177	2.13
level 3	10.845	0.249	2.30

- **Method comparison**

A comparison between CORMAY reagent (y) and commercially available assay (x) using 40 samples gave following results:

$$y = 0.96 x + 0.4532$$

$$R = 0.98 \quad (R - \text{correlation coefficient})$$

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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