HAEMOGLOBIN A_{1c} (HbA_{1c})

RX SERIES

INTENDED USE

A Haemoglobin A_{1c} test system is a device intended for the quantitative *in vitro* determination of Haemoglobin A_{1c} concentration in whole blood. This product is suitable for use on RX **series** instruments, which includes the RX **daytona** and RX **imola** analysers.

Cat. No.

HA 3830	HbA_{1c} R1: Antibody Reagent	3 x 14 ml
	HbA_{1c} R2: Agglutinator Reagent	3 x 14 ml
	R3: Haemoglobin Denaturant Reagent	3 x 50 ml
	Hb R1: Total Haemoglobin Reagent	3 x 28 ml

GTIN: 05055273203561

Supplementary Kit

HA 3450	Haemoglobin Denaturant Reagent **(Hb REAG DNT)**	2 x 50 ml

GTIN: 05055273203554

CLINICAL SIGNIFICANCE [1-9]

The measurement of HbA_{1c} is used in the long-term monitoring of diabetes mellitus. This assay should not be used in the diagnosis of diabetes mellitus or for day to day glucose monitoring.

Diabetes Mellitus is a disease associated with poor glycaemic control. Numerous clinical studies, including the Diabetes Control and Complications Trial, have shown that diabetes related complications may be reduced by the long term monitoring and tight control of blood glucose levels.

In the diabetic patient where blood glucose levels are abnormally elevated the level of HbA_{1c} also increases, the reason for this is that HbA_{1c} is formed by the nonenzymatic glycation of the N-terminus of the β-chain of haemoglobin A_0.

The level of HbA_{1c} is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 6-8 weeks. It is therefore, a long term indicator of diabetic control, whereas, the measurement of blood glucose is only a short term indicator.

PRINCIPLE

Both the concentration of HbA_{1c} and the concentration of total haemoglobin are measured. The reported HbA_{1c} result is calculated as a % of the total haemoglobin concentration.
The HbA_{1c} and total haemoglobin values generated in this assay are intended for use in the calculation of the HbA_{1c}/total haemoglobin ratio (%HbA_{1c}) and must not be used individually for diagnostic purposes.

In some methods, falsely elevated % HbA_{1c} results can be caused by the labile fraction of glycated haemoglobin (Schiff base). However, this assay is not affected by "labile HbA_{1c}" as the antibody used is specific for HbA_{1c} (a stable ketamine).

(a) Sample Pre-treatment

The first step of the procedure involves the pre-treatment of the whole blood sample. This lyses red blood cells and causes hydrolysis of the haemoglobin by the action of a protease enzyme in the Haemoglobin Denaturant Reagent.

(b) Determination of Total Haemoglobin[10]

The Total Haemoglobin reagent is used to determine the concentration of total haemoglobin. The method involves the conversion of all the haemoglobin derivatives into haematin in an alkaline solution of a non-ionic detergent as described by Wolf *et al* (1984).
The reaction is initiated by the addition of the pre-treated sample to the total haemoglobin reagent, resulting in a green solution. The conversion of different haemoglobin species into alkaline haematin with one defined absorption spectrum allows the endpoint measurement of total haemoglobin at 600 nm.

(c) Determination of HbA_{1c}

The determination of HbA_{1c} is based on a latex agglutination inhibition assay.

The agglutinator, which consists of a synthetic polymer containing multiple copies of the immunoreactive portion of HbA_{1c}, causes agglutination of latex coated with HbA_{1c} specific mouse monoclonal antibodies.

In the absence of HbA_{1c} in the sample, the agglutinator in the HbA_{1c} R2 Reagent and the antibody-coated micro particles in the HbA_{1c} R1 Reagent will agglutinate, resulting in an increase in absorbance.

The presence of HbA_{1c} in the sample will slow the rate of agglutination as it competes with the HbA_{1c} agglutinator for antibody binding sites on the latex.

Hence, the increase in absorbance is inversely proportional to the concentration of HbA_{1c} in the sample.

An increase in absorbance due to agglutination is measured at 700 nm and the extent of agglutination is used to calculate the concentration of HbA_{1c} from a Calibration Curve. The percentage HbA_{1c} is then calculated using the g/dl HbA_{1c} and Total Haemoglobin values.

SAMPLE COLLECTION AND STORAGE[11]

Venipuncture or capillary blood samples may be used.

Potassium EDTA, Ammonium Heparin or Lithium Heparin are recommended as anticoagulants.

Potassium-EDTA and Ammonium Heparin whole blood are stable at –70°C for 6 months or at +5°C for 2 weeks.

Frozen samples should be thawed at room temperature, mixed thoroughly prior to use and should not be refrozen.

Note. All blood samples should be mixed thoroughly immediately before assaying.

Stability of Pre-treated Sample

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

On storage, certain lysed samples have a tendency to precipitate. It is therefore good laboratory practice to mix/swirl samples prior to transferring to a system for analysis.

If overnight storage is required, we recommend that the lysed samples be stored at +2 to +8°C.

REAGENT COMPOSITION

Contents	
HbA_{1c} R1 : Antibody Reagent	
HbA_{1c} Antibody (mouse) coupled particles	<0.1% w/v
Bovine Serum Albumin	
Buffer	
Non ionic Surfactant	0.6% w/v
Proclin 150	0.1% w/v
pH 8.1	
HbA_{1c} R2 : Agglutinator Reagent	
HbA_{1c} hapten covalently attached to the polymer	
Bovine Serum Albumin	
Buffer	
Proclin 150	0.1% w/v
Non ionic Surfactant	0.2% w/v
pH 2.0	
R3 : Haemoglobin Denaturant	
Porcine Pepsin	
Buffer	
pH 2.4	
Hb R1 : Total Haemoglobin Reagent	
Sodium Hydroxide	0.4% w/v
Triton	2.5% w/v
Octylphenoxypolyethoxyethanol	2.5% w/v
pH 13	

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Total Hb Reagent contains sodium hydroxide which is caustic. In the event of accidental contact, flush affected area with large quantities of water and seek immediate medical attention.

Health and Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

All reagents are stable as supplied to expiry date when stored at +2 to +8°C, stored protected from extreme heat, light, or freezing.

Reagents should be mixed thoroughly and equilibrated to system temperature for approximately ½ hour prior to use on the system.

TOTAL Hb ASSAY

Reagent 1 = Total Hb Reagent

HbA_{1c} ASSAY

Reagent 1 = HbA_{1c} R1 : Antibody Reagent
Reagent 2 = HbA_{1c} R2 : Agglutination Reagent

MATERIALS PROVIDED

Hb Denaturant Reagent
Total Hb Reagent
HbA_{1c} R1 : Antibody Reagent
HbA_{1c} R2 : Agglutinator Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Haemoglobin A_{1c} Calibrator, Cat. No.	HA 3444
Haemoglobin A_{1c} Control Set, Cat. No.	HA 5072
Hb Denaturant Reagent	HA 3450
RX **series** Saline, Cat. No.	SA 3854

Glass micropipettes
Glass micropipette controller

PROCEDURE NOTES

Enter lot specific values based on those given in the HA 3444 calibrator insert. Calibrators do not require pre-treatment.

The Chemistry Parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the bar code cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Rx Support, Northern Ireland (028) 94451070.

SAMPLE PRETREATMENT

Mix **10 µl** of the whole blood sample with **400 µl** of haemoglobin denaturant reagent (1:41 dilution). We recommend the use of glass micropipettes to measure the whole blood sample volume.
Avoid foaming. Incubate for a minimum of 5 minutes at room temperature prior to testing.

CALIBRATION

HbA_{1c}

We recommend that this assay should be calibrated using Randox Haemoglobin A_{1c} Calibrator Series, levels 1 - 6.

Total Haemoglobin

We recommend that this assay should be calibrated using 0.9% NaCl solution as zero calibrator and Randox Haemoglobin A_{1c} Calibrator Series, level 1 only.

Note. Calibrators DO NOT REQUIRE PRETREATMENT.

The calibrators are referenced to an HPLC method for HbA_{1c} and the Drabkin's method for total haemoglobin.

QUALITY CONTROL

Randox Haemoglobin A_{1c} controls, Level 1 and Level 2 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories RX Support, Northern Ireland (028) 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

Note: Controls REQUIRE PRE-TREATMENT AFTER BEING RECONSTITUTED. Controls should be treated the same as patient samples and in accordance with kit or reagent being used.

CALCULATION

The calculation of the Haemoglobin A_{1c} concentration is generated using the following equation:
The percentage HbA_{1c} is calculated:

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

This value may be automatically calculated using the Calculated Test feature of the Rx Series analysers. See the relevant Rx Series operator manual for further information.

Conversion of NGSP Values to IFCC Equivalent Values[15]

The relationship between %HbA1c results from the NGSP network and the IFCC network was evaluated, and the following master equations developed:

NGSP = (0.0915 x IFCC) + 2.15
IFCC = (NGSP – 2.15) x 10.929

Results generated on **RX** series instruments are in NGSP equivalent units and can be converted to IFCC equivalent units using the equation above.

EXPECTED VALUES [6, 12-14]

Each laboratory should establish its own normal range.

Depending upon the assay used, HbA_{1c} is approximately 4 – 6% in nondiabetics, 6 – 8% in controlled diabetics, and can be as much as 20% in uncontrolled diabetics.

One hundred and twenty four apparently healthy males and females undergoing physical examination ("Normals") were tested for HbA_{1c} with this assay. The expected range of 4.5 – 6.2% and mean of 5.4% resulted.

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFICITY/INTERFERENCES [16]

The assay gives accurate and precise results for a range of total haemoglobin varying between 7 g/dl and 23 g/dl. Patients with severe anaemias (Total Haemoglobin < 7 g/dl) and those with polythemia (Total Haemoglobin > 23 g/dl) should not be assayed by this method.

Any case of shortened red cell survival such as haemolytic anaemia or other haemolytic disease, pregnancy, recent significant blood loss etc. will result in a decrease in % glycated haemoglobin.

Samples containing Haemoglobin variants S and C may result in up to a 40% increase of the expected HbA_{1c} value. Also, those containing variant F (>10%) may yield a lower value than expected. Hence, samples containing variants S,C and F (>10%) should not be compared to published normal or abnormal values.

A sample containing variant E was shown not to interfere.

Interfering Substance		Concentration with no Interference
Bilirubin	:	513 µmol/l (30 mg/dl)
Trigs	:	18 mmol/l (1600 mg/dl)
RF	:	2000 IU/ml
Acetylsalicylic acid	:	60 mg/dl
Sodium cyanate	:	50 mg/dl
Urea	:	83 mmol/l (500 mg/dl)

The labile fraction of glycated haemoglobin (Schiff base) does not interfere as the antibody is specific for the stable ketamine.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances[17]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

ANALYTICAL RANGE

LINEARITY (Total Haemoglobin only)

The analytical range for Total Haemoglobin is 7 g/dl to 23 g/dl.

ASSAY RANGE (HbA_{1c} only)

The range of this assay is approximately 0.25 - 2.4 g/dl.

These values are dependent on the lot specific values of the calibrators in use.

The analytical range for % HbA_{1c} is the concentration that corresponds to the level 6 Haemoglobin A_{1c} calibrator (2.40 g/dl HbA_{1c}, 17.1% HbA_{1c} at a total Haemoglobin of 14 g/dl).

Samples with values above 17.1% HbA_{1c} should not be diluted and results should be reported as >17.1% HbA_{1c}.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data was obtained using an RX **daytona** analyser at 37°C.

SENSITIVITY

The minimum detectable concentration of HbA_{1c} with an acceptable level of precision was determined as 0.25 g/dl.

PRECISION

Within run precision

	Level 1	Level 2	Level 3
Mean (%)	5.51	8.28	10.7
SD	0.14	0.55	0.44
CV(%)	2.5	6.67	4.00
n	20	20	20

Between run precision

	Level 1	Level 2	Level 3
Mean (%)	5.56	8.49	10.9
SD	0.21	0.31	0.52
CV(%)	3.77	3.63	4.82
n	20	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 0.96X
and a correlation coefficient of r = 0.98

40 patient samples were analyzed spanning the range 6.16 to 13.03%.

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The presence of a vertical bar in the margin indicates a technical update from the previous revision.

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