HbA1c

For discrete analyzers
Method: Immunoturbidimetry

Product code:	**1418-1057**	
Packaging:	**HbA1c**	**4 x 7.6 mL (R1) + 4 x 7.6 mL (R2)**
	Total Hemoglobin	**4 x 15 mL (R1)**

Store at 2 – 8°C
For *in vitro* use

INTENDED USE
Reagents for the quantitative determination of HbA1c (Hemoglobin A1c) in human whole blood, with BECKMAN COULTER AU400/480/600/600-IVD/640/680/2700/5400 and other discrete analyzers. For *in vitro* diagnostic use only.

CLINICAL SIGNIFICANCE
HbA1c is formed by non enzymatic glycosylation of the free amino-groups on the N-terminal of the β chain of hemoglobin A_0. The HbA1c level is proportional to the glucose level in the blood. Since glucose remains bound to red cells during their entire life cycle, measuring HbA1c provides an indication of the mean daily concentration of blood glucose for the past two months. The measurement of HbA1c is, therefore, considered to be a significant diagnostic tool for monitoring of diet control and therapies during diabetes treatment. The effective control of blood glucose levels is essential for the prevention of ketosis and hyperglycemia, and can possibly reduce the predominance and severity of secondary diabetic complications, such as retinitis, neuropathy, nephropathy and cardiac diseases.

METHOD PRINCIPLE
The concentrations of both HbA1c and Total Hemoglobin are determined. The HbA1c/Total Hemoglobin ratio is expressed as a percentage of HbA1c (HbA1c%). The HbA1c percentage test includes the use of four reagents: Total Hemoglobin R1, HbA1c antibodies reagent R1, Reagent R2 and Denaturant (code 1518-1059). In the preparation stage, total blood is mixed with the Denaturant (1:41) and incubated at room temperature for at least five minutes. Red blood cells are lysed and hemoglobin chains are hydrolyzed by the protease in the reagent.
Total Hemoglobin is determined through the transformation of all hemoglobin products into hematin in an alcaline solution of a non-ionized detergent. Adding pretreated sample into the Total Hemoglobin reagent results to a green solution, which is measured at 600 nm.
HbA1c is determined by a latex agglutination inhibition reaction. An agglutinator, composed of a synthetic polymer bearing multiple copies of immunoreactive HbA1c fractions, causes agglutination of latex particles bound to mouse monoclonal anti-HbA1c antibodies. In the absence of HbA1c in the sample, antibody bound latex particles in HbA1c R1 ant the agglutinator in HbA1c R2 will form insoluble complexes that result in increase of the optical absorbance of the suspension. HbA1c present in the sample reduces the rate of agglutination of antibody bound latex particles in HbA1c R1 and the agglutinator in HbA1c R2. The increase in absorbance at 700 nm is therefore inversely proportional to the HbA1c concentration in the sample.

METHOD LIMITATIONS
Refer to the book "Effects of Preanalytical Variables on Clinical Laboratory Tests" for possible interference of other pharmaceutical agents in this particular test. Interference of other agents is described in the "Clinical Guide to Laboratory Tests".
The reagent is designed especially for use with discrete analyzers. For chemistry protocols and further information contact the customer support unit at MEDICON.

REAGENT COMPOSITION
HbA1c R1
HbA1c antibody (rat) covered particles
Bovine Serum Albumin
Buffer pH 8.1
Surfactant: 0.6% non ionic detergent
Preservative 0.1% Proclin

HbA1c R2
HbA1c Hapten
Bovine Serum albumin
Buffer pH 2.0
Surfactant
Preservative 0.1% Proclin

Total Hemoglobin R1
Sodium hydroxide 0.4% pH 13
Surfactant: 0.7%
Non ionized detergent

WARNINGS – PRECAUTIONS
- This reagent is designed for in vitro diagnostic use. In vitro diagnostic reagents can be hazardous. They should be handled according to good laboratory techniques. Avoid inhalation and contact with eyes and skin.
- Samples should be considered as potentially infectious. Handle with special caution.
- All products of human origin have been deemed potentially biologically hazardous. International laws must be observed while handling them (gloves, lab coats)
- Dispose all waste according to national laws.
- MSDS is available by MEDICON upon request.

PREPARATION
Total Hemoglobin Reagent R1 and HbA1c Reagents R1 and R2 are ready to use and can be placed on the apparatus. Shake HbA1c R1 well before first use. The vials bear barcodes for automatic recognition by BECKMAN COULTER AU series analyzers.

REAGENT DETERIORATION
The reagents should not be used:
- When they do not exhibit the specified linearity or control values lie outside the acceptable range after recalibration.
- After prolonged exposure to sunlight or high temperature.

SHELF LIFE
Unopened, the reagent is stable at 2 – 8°C, up to the expiry date stated on their labels. Once opened, R1, R2 and Total Hemoglobin R1 remain stable for at least 1 month when stored on the analyzer.

SAMPLE
Whole blood, heparinized. Add 25μL of sample to 1000 μL Hemoglobin Denaturant. Incubate at room temperature for 5 minutes before use.
Samples (not pretreated) are stable for up to 1 week when stored at 25°C, 2 weeks when stored at 2 – 8°C and up to 6 months when frozen to ≤ –70°C. Hemolysed (pretreated) samples are stable for up to 8 hours when stored at room temperature, and up to 48 hours when stored at 2 – 8°C, if they are secured inside an airtight container.

CALIBRATION
MEDICON HbA1c calibrator (code: 1578-1058), is ready to use. Gently invert each vial several times before use to ensure homogenous mix.
To calibrate Total Hemoglobin reagent, only use Calibrator 1.
To calibrate HbA1c use Calibrators 1 to 6.
Calibration should take place every 1 month, or when there is a significant diversion from control limits. Recalibration should also be repeated after major maintenance is performed on the analyzer or after a critical part is replaced or when a significant shift in control values occurs.
Traceability: The calibrator HbA1c values are traceable to the IFCC2 HbA1c reference method through the IFCC HbA1c reference material. Total hemoglobin values assigned to the THb calibrator are traceable to the cyano-methemoglobin standard (BCR-522) of the Institute of Reference Materials and Methods (IRMM). The relationship between results of the NGSP network (DCCT alignment) and the IFCC network has been assessed and a Primary Equation for transforming IFCC units into DCCT/NGSP units has been developed.

PRIMARY EQUATION: DCCT/NGSP = (0.915 x IFCC) + 2.15

Defining the relationship between the two networks connects the IFCC traceable results to the clinically correct HbA1c results coming from the DCCT and from the UKPDS. The Primary Equation also provides those results with DCCT traceability to a reference method of higher class.
The percentage (%) HbA1c results stemming from this assay are automatically re-calculated by the instrument in units aligned with DCCT, using the Primary Equation [NGSP = (0.915 x IFCC) + 2.15] that is IFCC approved. Results are therefore expressed in units aligned with DCCT, as suggested by IFCC.

QUALITY CONTROL
Medicon provides the MEDICON HbA1c Control (code: 1578-1057) for quality control. Add 25μL of the control to 1000 μL of Hemoglobin Denaturant. Incubate at room temperature for 5 minutes before use. Control recovery should lie within the acceptable range. Results outside the acceptable range even after recalibration could be due to reagent deterioration, unsuitable storage conditions or control deterioration, instrument malfunction, or error during test procedure.

MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT
HbA1c Calibrator
Quality control material
Automated biochemistry analyzer
Common laboratory equipment

REFERENCE INTERVALS
Non diabetics: < 6%
For glycemic control of diabetics: < 7%
Reference values are based on current bibliography. Each laboratory should determine its own expected values as dictated by good laboratory practices.

SPECIAL PERFORMANCE CHARACTERISTICS
Lienarity
The assay is linear within measuring range 2.0 – 16%. When values exceed this range samples should be diluted accordingly.
Sensitivity
The lowest detectable level is estimated at 2.0%.
The lowest detection limit (LDL) is defined as the lowest concentration of analyte that is distinguishable from zero. A sample free of analyte is assayed 20 times within the assay and the HbA1c is calculated as the absolute mean plus three standard deviations.
Precision

Level (%)	Within Run CV%	Total CV%
4.98	0.75	1.32
6.56	1.25	1.18

Precision is estimated on two concentration levels of analyte according to NGSP protocol (20 consecutive days, 2 runs per day, 2 repeats per run).
Interferences Criterion: recovery within ±10% of target value

Ascorbic acid	Insignificant up to 50 mg/dl
Bilirubin	Insignificant up to 50 mg/dl
Triglycerides	Insignificant up to 2000 mg/dl
Carbamylic hemoglobin	Insignificant up to 7.5 mmol/L
Acetylic hemoglobin	Insignificant up to 2000 mmol/L

Correlation: A comparison was performed between this reagent and another commercially available product of the BECKMAN COULTER AU series. The results were as follows:
Y=1.092X – 0.147 R=0.995 N=97 Sample range = 5.1 – 11.5 %

BIBLIOGRAPHY

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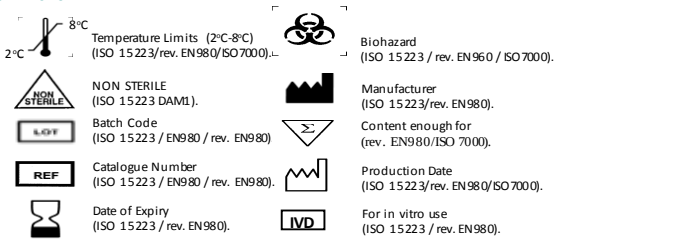
SYMBOLS


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Version 02 Last updated: 22/02/2010