



HbA1c APT (Hemoglobin A1c, Whole Blood Application)

<u>OSR61177</u>	HbA1c APT	2 x 19 mL	R1
		2 x 19 mL	R2
	Total Hemoglobin APT	2 x 37.5 mL	R1
	HbA1c APT Denaturant	2 x 55 mL	R1

Intended Use

Immuno-inhibition test for the quantitative determination of HbA1c (Hemoglobin A1c), in human whole blood, on the Beckman Coulter AU680 with whole blood automated pre-treatment (APT) capability only.
For *in vitro* diagnostic use only.

The absolute HbA1c and Total Hemoglobin (THb) values generated as part of the HbA1c assay are intended for use in the calculation of the HbA1c/Total Hemoglobin ratio, and must not be used individually for diagnostic purposes.

Summary¹

HbA1c is formed by the non-enzymatic glycation of free amino groups at the N-terminus of the β -chain of hemoglobin A₀. The level of HbA1c is proportional to the level of glucose in the blood. As the glucose remains bound to hemoglobin in the red cell throughout its life cycle of the cell, measurement of HbA1c provides an indication of the mean daily blood glucose concentration over the preceding three months. Measurement of HbA1c is, therefore, considered to be an important diagnostic tool in the monitoring of dietary control and therapeutic regimes during the treatment of diabetes. Effective control of blood glucose levels is important in the prevention of ketosis and hyperglycemia, and may reduce the prevalence and severity of late diabetic complications such as retinopathy, neuropathy, nephropathy, and cardiac disease.

Methodology

The concentrations of both HbA1c and Total Hemoglobin are determined. The HbA1c/Total Hemoglobin ratio is expressed as percentage HbA1c (%HbA1c). The assay for percent HbA1c, involves the use of four reagents: Total Hemoglobin APT R1, HbA1c APT R1 antibody reagent, HbA1c APT R2 agglutinator reagent, and HbA1c APT Denaturant.

Whole blood is pretreated automatically on board the analyzer. The red blood cells are lysed and the hemoglobin chain is hydrolysed by the protease present in the reagent.

Total Hemoglobin is measured via the conversion of all hemoglobin derivatives into alkaline hematin in the alkaline solution of a non-ionic detergent. Addition of the pre-treated blood sample to the Total Hemoglobin reagent results in a green solution, which is measured at 600nm.

HbA1c is measured in a latex agglutination inhibition assay. An agglutinator, consisting of a synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c, causes agglutination of latex coated with HbA1c specific mouse monoclonal antibodies. In the absence of HbA1c in the sample, the antibody-coated microparticles in the HbA1c APT R1 and the agglutinator in the HbA1c APT R2 will agglutinate. Agglutination leads to an increase in the absorbance of the suspension. The presence of HbA1c in the sample results in a decrease in the rate of agglutination of the HbA1c APT R1 and the agglutinator in the HbA1c APT R2. The increase in absorbance is, therefore, inversely proportional to the concentration of HbA1c in the sample. The increase in the absorbance is measured at 700nm.

System Information

For AU680 Beckman Coulter Analyzer.

Reagents

HbA1c APT R1

HbA1c Antibody (mouse) coupled particles
Bovine Serum Albumin
Buffer pH 8.1
Surfactant: 0.6% Non-ionic detergent
Preservative: 0.1% Proclin

HbA1c APT R2

HbA1c Hapten
Bovine Serum Albumin
Buffer pH 2.0
Surfactant
Preservative: 0.1% Proclin

Total Hemoglobin APT R1

Sodium hydroxide 0.4%; pH 13
Surfactant: 0.7% Non-ionic detergent

HbA1c APT Denaturant R1

Porcine Pepsin
Buffer pH 2.4
Preservative

Precautions

1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Irritating to eyes and skin.
4. Exercise the normal precautions required for handling all laboratory reagents.
5. Dispose of all waste material in accordance with local guidelines.
6. Safety data sheet available for professional user on request.

Preparation of Reagents

Total Hemoglobin APT R1 and HbA1c APT R2 are ready for use, and can be placed directly on board the instrument. No preparation is required. HbA1c APT R1 and HbA1c APT Denaturant R1 should be mixed by inversion 5 – 10 times before placing on board the instrument and at weekly intervals thereafter.

Do not mix reagents between kit lots. Do not top up reagent vials.

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Storage and stability²

1. The unopened reagents are stable, up to the stated expiry date when stored at 2 – 8°C.
2. Opened bottles of reagent are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration

Visible signs of microbial growth, gross turbidity, precipitate, or change in color in the HbA1c APT, Total Hemoglobin APT or HbA1c APT denaturant reagents may indicate degradation and warrant discontinuation of use.

Specimen Collection and Preparation²

K₂-EDTA or NH₄-heparinized whole blood.

HbA1c Calibrators (Cat # ODR3032) do not require pre-treatment. Please note only Hemoglobin Denaturant Cat # OSR61177 can be used with this method.

Whole blood samples should be mixed by inverting 5 – 10 times before placing on board the instrument. Samples should be analyzed within 60 minutes of mixing.

Sample Storage and Stability²

Samples (non-pretreated) are stable up to 1 week when stored at 25°C, 2 weeks when stored at 2 – 8°C, and up to 6 months when frozen at ≤ -70°C.

Reconstituted control material should not be pre-treated with HbA1c APT Denaturant, but should be placed on-board the instrument for on-board denaturation.

Interfering Substances

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 30 mg/dL Bilirubin
Lipemia: No significant interference up to 500 mg/dL Intralipid[®]*
Triglyceride: No significant interference up to 1600 mg/dL Triglyceride

*Intralipid[®] is a 20% IV fat emulsion used to emulate extremely turbid samples, (manufactured by KabiVitrium Inc.).

Rheumatoid factor (RF) up to 2000 IU/mL, acetylsalicylic acid (60 mg/dL), sodium cyanate (50 mg/dL) and urea (500 mg/dL), do not interfere with this assay.

Refer to Young³ for further information on interfering substances.

Procedure

Total Hemoglobin and HbA1c tests must be performed on each sample and control which have been pre-treated on-board the analyzer. A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials provided

Total Hemoglobin Reagent
HbA1c Reagent
HbA1c Denaturant

Materials Required But Not Provided

AU680 Analyzer
HbA1c Calibrator Cat # ODR3032

Stability of Final Reaction Mixture

The Beckman Coulter AU analyzers automatically compute every determination at the same time interval.

Calibration^{4,5,6}

The frequency of calibration for the HbA1c procedure is every 14 days. Calibration of this HbA1c procedure is accomplished by use of the HbA1c Calibrator (Cat # ODR3032). For calibration procedure, please refer to HbA1c Calibrator (Cat # ODR3032) Instructions for Use.

HbA1c Calibrator (Cat # ODR3032) is a 6 point calibrator. Calibrator 1 is used for calibration of the Total Hemoglobin assay. Calibrators 1 to 6 are used for calibration of the HbA1c assay.

Recalibration of the test is required when any of the following conditions exist:

Change in reagent bottle or significant shift in control values.

Preventative maintenance was performed on the analyzer.

A critical part was replaced.

Following calibration, the resulting curve should be visually reviewed, on the Beckman Coulter AU analyzer, for acceptability.

Calibrator HbA1c values have been assigned by immunoturbidimetry using HbA1c (Cat # OSR61177) and are traceable to the IFCC HbA1c reference method via IFCC reference material. Total Hemoglobin values are assigned to the THb Calibrator are traceable to the IRMM Hemoglobin Cyanide Standard (BCR – 522). Any instrument or reagent modification may invalidate the assigned value.

%HbA1c values are traceable to the Diabetes Control and Complications Trial (DCCT) via the Master Equation developed by the National Glycohemoglobin Standardization Program (NGSP) and International Federation of Clinical Chemistry (IFCC).

The relationship between results from the NGSP network (DCCT aligned) and the IFCC network has been evaluated, and a Master Equation has been developed for interconversion of results from IFCC to NGSP units.

Master Equation

$$\text{NGSP} = (0.915 \times \text{IFCC}) + 2.15$$

Definition of the relationship between the two networks links IFCC-traceable results to clinically meaningful HbA1c results from the DCCT and the United Kingdom Prospective Diabetes Study (UKPDS). The Master Equation also provides these DCCT results with traceability to a higher order reference method.

Quality Control

During operation of the Beckman Coulter AU analyzer, at least two levels of control material such as HbA1c Control (Cat # ODC0022) should be tested a minimum of once a day. In addition, these controls should be tested after every calibration event, with each new bottle of reagent, and after specific troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results

HbA1c reagents should be set up according to the method parameters. Calibrator values are assigned in IFCC units. %HbA1c results are automatically recalculated to DCCT aligned units by the instrument using the NGSP/IFCC approved Master Equation. This calculation is pre-programmed into the analyzer for this test. The result will be automatically printed out for %HbA1c at 37°C. THb and HbA1c are used for determination of %HbA1c. %HbA1c is calculated automatically by the analyzer using the following formula: $(A/B) \cdot a + b$, where $A = \text{HbA1c}$, $B = \text{THb}$, $a = 91.5$, and $b = 2.15$.

Limitations of The Procedure²

Specimens with greatly elevated Erythrocyte Sedimentation Rate (ESR) may result in inaccurate HbA1c values. For such samples the analysis should be performed immediately after mixing. Shortened red cell survival time will reduce the exposure of red cells to glucose, with a resultant decrease in %HbA1c values. Percentage HbA1c results are therefore not reliable where red cell survival time is reduced. Causes of reduced red cell survival time include hemolytic anemia, or other hemolytic disease, significant blood loss and pregnancy. Samples containing hemoglobin variants S and C may produce up to a 40% elevation of the expected HbA1c value in this assay. Samples containing > 10% of hemoglobin F may yield a lower than expected result with this test. HbA1c results obtained by this test method for blood samples containing Hb variants S, C and F (>10%) should not be compared to published normal or abnormal values. A sample containing Hemoglobin E was shown not to interfere with this test. The labile fraction of glycated hemoglobin (Schiff base attachment of glucose to HbA or HbA1c) does not affect the assay result due to the specificity of the antibody for the stable ketoamine. As with any chemical reaction, users should be aware of the possible effect on results due to unknown interferences from medication or endogenous substances. For diagnostic purposes, the HbA1c results should always be assessed in conjunction with other available information e.g. patient's medical history, clinical status and results of other tests.

Expected Values^{2,5}

Adults: 4.0 – 6.2 %HbA1c

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the HbA1c reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Dynamic Range

Total Hemoglobin
The dynamic range for Total Hemoglobin is 7 – 23 g/dL (4.4 – 14.3 mmol/L)

HbA1c

The dynamic range of this assay extends from 3.2 %HbA1c to the concentration of Calibrator 6 which approximately corresponds to 14.5 %HbA1c at a total hemoglobin level of 14.5 g/dL (9 mmol/L). Samples exceeding the upper limit of linearity should not be diluted, but instead should be reported as >14.5%.

Precision⁷

Estimates of precision, based on CLSI recommendations are consistent with typical performance. The within run precision is $\leq 3\%$ CV and the total precision is $\leq 4\%$ CV on the AU680 instrument. Assays of control material were carried out and data reduced following CLSI guidelines above.

The following data was obtained on an AU680 analyzer using 3 pools analyzed over 20 days.

AU680

N = 80	Within run		Total	
	Mean, %	SD	CV%	CV%
5.20	0.02	0.43	0.05	0.95
7.34	0.05	0.73	0.07	0.92
11.17	0.06	0.56	0.10	0.88

Method Comparison

Patient samples were used to compare this HbA1c APT OSR61177 assay (Method Y) on the AU680 against the HbA1c OSR6192 assay on the AU640 (Method X). Results of linear regression analysis were as follows:

Y Method	AU680
	X Method
Slope	1.026
Intercept	0.437
Correlation Coeff.	0.966
No. of Samples	105
Range (%HbA1c)	4.80 – 13.45%

Sensitivity

The lowest detectable level on AU680 analyzer was calculated to be the following:

HbA1c 0.02 g/dL (0.01mmol/L)
THb 0.08 g/dL (0.05mmol/L)

The lowest detectable level represents the lowest measurable level of HbA1c that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

Technical Assistance

For technical assistance contact the Beckman Coulter Technical Support Center at (800) 223-0130.

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References

1. Niederau CM, Reinauer H Glycohemoglobins In: Thomas L, ed. Clinical Laboratory Diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main:TH-Books Verlagsgesellschaft mbH, 1998: 142-148.
2. Data on file at Beckman Coulter Inc.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Jeppsson J-O, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C. Approved IFCC reference method for the measurement of HbA1c in Human Blood. Clin Chem Lab Med 2002; 40(1): 78-89.
5. Hoelzel W, Weykamp C, Jeppsson J-O, Miedema, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Theinpoint L, Umemoto M, Wiedmeyer H-M. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardisation Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. Clin Chem 2004; 50(1): 166-174.
6. DCCT 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.
7. CLSI/NCCLS. Evaluation of precision performance of clinical chemistry devices; approved guideline. CLSI/NCCLS document EP5-A; 1999.

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