HbA1c (Hemoglobin A1c)

B00389	**2 x 37.5 mL**		**HbA1c R1**
	2 x 7.5 mL		**HbA1c R2**
	2 x 34.5 mL		**Total Hemoglobin R1**
	5 x 2 mL	**LYO**	**HbA1c Calibrator (Levels 1-5)**

Intended Use

The HbA1c (Hemoglobin A1c) reagent, when used in conjunction with Beckman Coulter Systems, HbA1c Calibrators, and SYNCHRON and AU Hemolyzing Reagent, is intended for the quantitative determination of hemoglobin A1c concentration in human whole blood. 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.

Summary[1,2,3,4,5,6,7,8]

Measurement of hemoglobin A1c is accepted as a method to measure long-term glucose control in patients with diabetes mellitus (a chronic disorder associated with disturbances in carbohydrate, fat and protein metabolism and characterized by hyperglycemia). Determination of HbA1c provides an important tool for monitoring the efficiency of dietary control and therapy during treatment of diabetes mellitus. Long term treatment of the disease emphasizes control of blood glucose levels in preventing the acute complications of ketosis and hyperglycemia. In addition, long term complications such as retinopathy, neuropathy and cardiovascular disease can be minimized if blood glucose levels are effectively controlled.
The process of conversion from hemoglobin A to hemoglobin A1c depends on the blood glucose concentration. Since the average life of a red blood cell is 120 days, measurement of hemoglobin A1c can reflect the mean daily blood glucose concentration over the preceding two to three months and provides a much better indication of glycemic control than blood or urinary glucose determinations.

Methodology

The HbA1c assay (B00389) involves the use of four reagents: Total Hemoglobin R1, HbA1c R1, HbA1c R2, and Hemolyzing Reagent (sold separately as Cat. No. 472137). In a pre-treatment step, whole blood is mixed with the Hemolyzing Reagent in a 1:100 dilution and the resultant hemolysate is used. Tetradecyltrimethylammonium bromide (TTAB) in the hemolyzing reagent eliminates interference from leukocytes.
The concentrations of both HbA1c and Total Hemoglobin are determined. The HbA1c/Total Hemoglobin ratio is expressed either as mmol/mol (IFCC) or %HbA1c (DCCT/NGSP).
Total Hemoglobin Reagent is used to measure total hemoglobin concentration by a colorimetric method. Change in absorbance is measured at 570/660 nm.
HbA1c reagent is used to measure hemoglobin A1c concentration by a turbidimetric immunoinhibition method. In the reaction, hemoglobin A1c antibodies combine with HbA1c from the sample to form soluble antigen-antibody complexes. Polyhaptens from the reagent then bind with the excess antibodies and the resulting agglutinated complex is measured turbidimetrically. Change in absorbance is measured at 340/700 nm.

System Information

For AU400/AU480, AU640/640e/680,AU2700 /AU5400 and AU5800 Beckman Coulter Analyzers.

Contents, Reagent Composition

HbA1c Calibrator
Hemolysate (human and sheep)
0.9% tetradecyltrimethylammonium bromide
Non-reactive chemicals necessary for optimum system performance

HbA1c R1 Antibody Reagent		HbA1c R2 Polyhapten Reagent	
Anti-Human HbA1c Antibody (sheep)	≥ 0.5mg/mL	HbA1c Polyhapten	≥ 8 µg/mL
MES (2-morpholino-ethanesulphonic acid) Buffer	0.025 mol/L	MES (2-morpholino-ethanesulphonic acid) Buffer	0.025 mol/L
TRIS (tris(hydroxymethyl) aminomethane) Buffer, Ph 6.2	0.015 mol/L	TRIS (tris(hydroxymethyl)aminomethane) Buffer, pH 6.2	0.015 mol/L
Non-reactive chemicals necessary for optimum system performance			

Total Hemoglobin

Total Hemoglobin R1	
Phosphate Buffer, pH 7.4	0.02 mol/L
Non-reactive chemicals necessary for optimum system performance	

Materials needed but not supplied with reagent kit

SYNCHRON and AU Systems Hemolyzing Reagent (for use in sample preparation); Cat. No. 472137.
0.9% Saline solution.
At least two levels of control material.
0.1M NaOH (Analar Grade (AR)).

Precautions and Warnings[9]

Hazard Warnings and Risk Phrases:
WARNING: POTENTIAL BIOHAZARDOUS MATERIAL.
The calibrator is manufactured from human material; each donor used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV-1/2 and HCV as well as for hepatitis B surface antigen and was not repeatedly reactive. Because no test method can offer complete assurance that HIV-1/2, HCV, hepatitis B virus or other infectious agents are absent from biological materials, this product should be handled at the Biosafety Level 2 as recommended for any infectious human serum or blood specimen in the Centers for Disease Control and Prevention/National Institutes of Health manual, *Biosafety in Microbiological and Biomedical Laboratories.*

Xi; Irritant R36/38 Irritating to eyes and skin
Safety phrases:
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Exercise the normal precautions required for handling all laboratory reagents.
Dispose of all waste material in accordance with local guidelines.
Refer to Safety Data Sheets for further information.

Reagent Preparation

Total Hemoglobin R1 and HbA1c R1 and R2 are ready for use, and can be placed directly on board the analyzer.
Bring Hemolyzing Reagent to room temperature prior to use.

Reagent Storage and stability

1. The unopened reagents are stable, up to the stated expiration 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.

DO NOT FREEZE. Once opened, the SYNCHRON and AU Systems Hemolyzing Reagent is stable until the expiration date printed on the bottle label when stored and capped at +2°C to +8°C.

Indications of Deterioration

Visible signs of microbial growth, turbidity, precipitate or any change in color in the HbA1c reagent may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation

K_2-EDTA, K_3-EDTA, Li-Heparin or Na-Heparin whole blood (freshly drawn blood treated with EDTA is the preferred specimen). Bring the Hemolyzing Reagent to room temperature before use. Pre-treat samples and controls by dilution of whole blood with Hemolyzing Reagent in a ratio of 1:100 (for example 10 µL of sample or control plus 1000 µL of Hemolyzing Reagent Cat. No. 472137). Mix thoroughly, avoid foaming and assay the hemolysate after hemolysis is complete (allow at least 1 minute for Hemolysis). **Please note that only SYNCHRON and AU Systems Hemolyzing Reagent Cat. No. 472137 can be used with this method.**

Specimen storage and stability

Samples (non-pretreated) are stable up to 8 hours when stored at 25°C, 7 days when stored at 2…8°C and up to 3 months when frozen at -20°C. Whole blood samples are stable for 18 months at -70°C.[7] Frozen samples should be thawed only once.
Hemolyzed (pre-treated) samples are stable up to 4 hours when stored at 15…25°C, up to 24 hours when stored at 2…8°C, if stored in a sealed container.
Note: All hemolyzed samples should be mixed thoroughly immediately prior to assay.
Each laboratory should evaluate sample handling procedures to avoid variable results.

Test Procedure

Total Hemoglobin and HbA1c tests must be performed on each pre-treated sample and control. Refer to the appropriate User's Guide and the accompanying Instrument Setting Sheets for analyzer-specific assay instructions.

Calibration

Calibrators are included in the kit.

Calibrator value assignment
Refer to table of assigned values included in the kit.

NOTE: Calibrators are lot-specific and should not be interchanged. Calibrators DO NOT require pre-treatment with hemolyzing reagent prior to assay.
Total Hemoglobin: Two Point Calibration
0.9% Saline and HbA1c Calibrator Level 2 are used for calibration of the Total Hemoglobin assay.

HbA1c: Multi-point Calibration.
HbA1c Calibrator Levels 1 to 5 are used for calibration of the HbA1c assay.

Calibrator Preparation
1. Carefully remove the cap and stopper from each bottle, avoiding any loss of lyophilized material.
2. Add 2.0 mL of sterile deionised water at 15…25°C to the lyophilized material using a calibrated volumetric pipette.
3. Replace the caps and stoppers on each bottle. Care must be taken to ensure that the caps and stoppers are matched to the correct bottle.
4. Dissolve the contents completely by gently mixing for 30 minutes. Vortex each bottle for 5 seconds at medium speed. Avoid foaming. Ensure that all lyophilized material is reconstituted.
5. Record the date the calibrator was reconstituted on each bottle label.

Calibrator storage and stability
Unopened, the calibrators should be stored at 2 … 8°C until the expiration date printed on the calibrator bottle.
Reconstituted calibrators are stable for 8 hours stored at 15…25°C or 30 hours stored at 2…8°C until the expiration date is exceeded.
Calibrators that are aliquoted immediately after reconstitution and stored at -20°C are stable for 30 days, as long as the expiration date of the un-reconstituted calibrator is not exceeded. Frozen calibrators should be thawed only once. After thawing, vortex each bottle for 5 seconds at medium speed. Avoid foaming.
These calibrators should only be used in conjunction with the reagent system described here on Beckman Coulter AU Systems.

Calibration Stability
Recalibrate the assay every 14 days, or when the following occur:
1. Change in reagent lot or significant shift in control values;
2. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

Following calibration, the resulting curve should be visually reviewed on the Beckman Coulter AU Analyzer for acceptability using the software options to access the Calibration Monitor.

Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

Traceability[10,11,12,13,14,15,16]

The calibrator HbA1c values are traceable to the IFCC HbA1c reference method via IFCC HbA1c reference material. 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 (mmol/mol) to NGSP (%) units.

MASTER EQUATION

$$\text{NGSP} = (0.0915 \times \text{IFCC (mmol/mol)}) + 2.15$$

The 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.

Results

To report %HbA1c in NGSP units, this has to be defined as a CALCULATED TEST in the INTER TEST or CALCULATED TEST menu. Enter the formula (A/B)*a + b, where A=HbA1c, B=THb, a=91.5, b=2.15.
To report HbA1c in IFCC units (mmol/mol), this has to be defined as a CALCULATED TEST in the INTER TEST or CALCULATED TEST menu. Enter the formula (A/B)*a, where A=HbA1c, B=THb, a=1000.
Note: HbA1c and THb must be selected in the same units.

Quality Control

At least 2 levels of control material should be analyzed. Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed.
The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.
If any trends or sudden shifts in values are detected, review all operating parameters.
Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

Reference Interval[17,18,19,20]

Adults: 4.0 – 6.0% (NGSP)
20 – 42 mmol/mol (IFCC units)

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. Results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Specific Performance Characteristics

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values

Analytical Range

Total Hemoglobin
The analytical range for Total Hemoglobin is 3.7-13.0 mmol/L (6-21 g/dL). When the result for Total Hemoglobin is outside the analytical range an "F" or "G" flag is generated and the calculated % HbA1c should not be reported. Settling of the red cells before the aliquot is taken for pre-treatment may cause an elevated Total Hemoglobin result. Samples exceeding the upper limit of Total Hemoglobin may be mixed well and the analyses repeated on a freshly hemolyzed sample.

HbA1c
The analytical range of this assay extends from 0.19 mmol/L (0.3 g/dL) to the concentration of Calibrator 5.
If the HbA1c concentration is outside the analytical range an "F" or "G" flag is generated and the calculated % HbA1c should not be reported.
Samples exceeding the upper limit of the analytical range for HbA1c should not be diluted, but instead should be reported as "% HbA1c > 15%" or " HbA1c>140 mmol/mol".

%HbA1c
The reportable range for the calculated HbA1c is 20 – 140 mmol/mol HbA1c (IFCC) and 4 – 15 % HbA1c (NGSP), based on a typical Calibrator 5 value of 1.36 mmol/L (2.19g/dL), at a total hemoglobin of 9.6 mmol/L (15.5 g/dL).
Note: a calculated result may be outside the reportable range based on either the Total Hemoglobin or the HbA1c result being outside of their respective analytical ranges.

Precision[21]

Correctly operating AU Systems should exhibit precision values less than or equal to the following:

TYPE OF IMPRECISION	SAMPLE TYPE	%HbA1c (NGSP) % CV
Within Run	Whole Blood Hemolysate	4.0
Total	Whole Blood Hemolysate	4.0

Estimates of imprecision, based on CLSI recommendations are consistent with typical performance. Comparative performance data for the AU2700 system evaluated using the CLSI approved guideline EP5-A2 appears in the table below. Each laboratory should characterize their own instrument performance for comparison purposes.

The following data was obtained using 3 pools analyzed over 20 days.

TYPE OF IMPRECISION	SAMPLE TYPE	No. of Data Points	Mean %HbA1c	CV%	SD (%HbA1c)
Within run	Hemolysate Control Pool 1	80	5.3	1.44	0.08
	Hemolysate Control Pool 2	80	7.4	1.03	0.08
	Hemolysate Control Pool 3	80	9.4	1.03	0.10
Total	Hemolysate Control Pool 1	80	5.3	2.07	0.11
	Hemolysate Control Pool 2	80	7.4	1.84	0.14
	Hemolysate Control Pool 3	80	9.4	1.68	0.16

Sensitivity[22]

The limit of blank (LoB) and limit of detection (LoD) were determined in accordance with the CLSI EP17-A guideline. The LoB is calculated from n ≥ 60 measurements of an analyte-free sample, and corresponds to the concentration below which analyte-free samples are found with 95% confidence. The Limit of Detection (LoD) corresponds to the sample concentration above the LoB which is detectable with 95% confidence.

Total Hemoglobin
LoB = 0.05 mmol/L (0.09 g/dL)
LoD = 0.10 mmol/L (0.16 g/dL)

HbA1c
LoB = 0.12 mmol/L (0.19 g/dL)
LoD = 0.13 mmol/L (0.22 g/dL)

Method Comparison[23]
Patient samples were run in singlicate to compare this HbA1c (B00389) assay against OSR6192 on the AU680 analyzer. Results of Deming regression analysis were as follows:

Slope = 1.036	Intercept = -0.3821	r = 0.9968	n = 116	Sample range = 4.6 – 12.0% HbA1c

Patient samples were run in singlicate to compare this HbA1c (B00389) assay on the AU680 analyzer against HbA1c (650262) on the DXC800 analyzer. Results of Deming regression analysis were as follows:

Slope = 0.901	Intercept = 0.3140	r = 0.9941	n = 130	Sample range = 4.9 – 14.2% HbA1c

Anticoagulant Test Results
The following anticoagulants were assessed by Deming regression analysis with paired EDTA and heparin whole blood samples. Values of K_2-EDTA (X), ranging from 4.7 % HbA1c to 14.7 % HbA1c, were compared with values for K_3-EDTA, lithium heparin whole blood and sodium heparin whole blood (Y), yielding the following results:

ANTICOAGULANT	CONCENTRATION OF ANTICOAGULANT TESTED	DEMING REGRESSION ANALYSIS
K_3-EDTA	1.8 mg/mL	Y = 0.995X + 0.023 ; r = 0.999
Lithium Heparin	17 I.U./mL	Y = 0.990X + 0.039 ; r = 0.999
Sodium Heparin	17 I.U./mL	Y = 0.998X + 0.007 ; r = 0.999

Interfering Substances[24,25,26]
Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:
Icterus: Interference ≤ 6% up to 513 µmol/L (30 mg/dL) Bilirubin
Lipemia: Interference ≤ 7% up to 500 mg/dL Intralipid®-a
Ascorbic Acid: Interference ≤ 6% up to 50 mg/dL Ascorbic Acid
Rheumatoid factor (RF): Interference ≤ 6% up to 1000 IU/mL Rheumatoid Factor
Intralipid®-a is a registered trademark of Fresenius Kabi AB., Uppsala, Sweden.
Refer to references for further information on interfering substances.

Specificity[24,27,28,29,,30,31,32]
The HbA1c test shows no cross-reactivity with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin and glycated albumin.
No significant effect of HbS, HbD, HbE, HbC, and HbF up to 10% was observed with this assay. Glycated HbF is not detected by the HbA1c assay as it does not contain the glycated β-chain. However, HbF is measured in the THb assay.
Samples containing >10% HbF may result in lower than expected HbA1c results.
No significant effect (≤ 10%) of labile glycated hemoglobin (up to 2000 mg/dL, 5 hours at +37°C) was observed with this assay.

Limitations[17,21,30,31,33]

1. This assay is designed only for the measurement of mmol/mol HbA1c (IFCC) and %HbA1c (NGSP). Individual results for Hb and HbA1c concentration should not be reported.
2. Do not use this test for the diagnosis of diabetes mellitus. Performance characteristics for this use have not been determined.
3. This assay is not useful in judging day-to-day glucose control and should not be used to replace daily home testing of glucose.
4. Shortened red cell survival time will reduce the exposure of red cells to glucose, with a resultant decrease in HbA1c values. Causes of reduced red cell survival time include hemolytic anemia, or other hemolytic diseases, significant blood loss, blood transfusions, iron deficiency and pregnancy. Caution should be exercised when interpreting the HbA1c results from patients with these or other conditions affecting red cell survival time, and when the total hemoglobin is 5.6 mmol/L (<9 g/dL).
5. Sample carryover from the hemolysate can affect subsequent Urinary/CSF Protein (OSR6x70) results, and reagent carryover from HbA1c (B00389) can adversely affect Urine Amphetamines (OSR6323) and Urine THC (OSR6322) assays. Please refer to AU Contamination Avoidance Parameters for guidance.
6. Prior to weekly maintenance a mandatory additional W2 cycle using 0.1M NaOH is required when running HbA1c from standby, in batch mode or random access mode.
   When running the HbA1c assay in **batch mode from standby**, a mandatory W2 using 0.1M NaOH followed by a W2 using 1M HCl and photocal, is required after every 4th batch of samples, where the analyzer is returned to standby between batches. This represents, potentially, 4 occupations of the same cuvette. Batch sample capacity is based on the cuvette capacity of the specific analyzer in use.
   When running the HbA1c assay in **random access mode** (i.e. together with other assays) additional parameters are necessary due to increased risk of cuvette coating by this assay. Please refer to AU Contamination Avoidance Parameters for guidance.
   If unacceptable drift or imprecision is observed in any Quality Control results or any calibration failures are observed, an additional W2 cuvette cleaning using 0.1M NaOH followed by 1M HCl and photocal, is recommended.

Note 1: Weekly maintenance should be carried out according to AU User Guides.
Note 2: As with any chemical reaction, users should be aware of the possible effect on results due to unknown interferences from medication or endogenous substances.

Setting Sheet Footnotes
\# User defined
† HbA1c Calibrators included in the kit.
* Values set for working in mmol/L. To convert to g/dL, multiply by 1.6125.
** Concentration of 0.19 mmol/L and Calibrator 5.
¥ For determination of % HbA1c (NGSP units), the THb and HbA1c tests are used. A third test % HbA1c must also be entered in the general tests and the calculated tests of the test section menu (no settings required). ***Set this test as CALCULATED TEST in the INTER TEST menu/ ****Set this test in common Test Parameter test name as CALCULATED TEST. In Specific Test Parameters Calculated Test, enter the formula (A/B)*a+b, where A= HbA1c, B=THb, a=91.5 and b=2.15. To report results as mmol/mol HbA1c (IFCC units), enter the formula (A/B)*a, where A=HbA1c, B=THb, a=1000.

Note: HbA1c and THb must be selected in the same units.
*** For AU400, AU640/640^e and AU2700/AU5400 Beckman Coulter Analyzers.
**** For AU480,AU680 and AU5800 Beckman Coulter Analyzers.

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