510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k120199

B. Purpose for Submission:

New Device

C. Measurand:

Glycosylated Hemoglobin A1c (HbA1c)

D. Type of Test:

Quantitative turbidimetric inhibition immunoassay

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

AU® Systems HbA1c (Hemoglobin A1c) Test System

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LCP	Class II	864.7470	Hematology, 81
JIT	Class II	862.1150	Chemistry, 75

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for 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.

The HbA1c Calibrators are an in vitro diagnostic product for the calibration of the hemoglobin A1c (HbA1c) method on the AU clinical chemistry systems.

Measurement of hemoglobin A1c is accepted as a method to measure long-term glucose control in patients with diabetes mellitus.

3. Special conditions for use statement(s):

- Prescription use only.
- 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.
- Do not use this test for the diagnosis of diabetes mellitus. Performance characteristics for this use have not been determined.
- This assay is not useful in judging day-to-day glucose control and should not be used to replace daily home testing of glucose.
- 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 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 <9 g/dL (5.6 mmol/L).
- Caution should be exercised when interpreting the HbA1c results from patients with hemolytic disease or other conditions characterized by shortened erythrocyte survival, acute blood loss, and iron deficiency.
- As with any chemical reaction, users should be aware of the possible effect on results due to unknown interferences from medication or endogenous substances

4. Special instrument requirements:

For use on the Beckman Coulter AU480/AU680/AU2700 Clinical Chemistry Analyzers

I. Device Description:

The AU® Systems HbA1c (Hemoglobin A1c) Test System involves the use of four reagents: Total Hemoglobin R1, HbA1c R1, HbA1c R2, and Hemolyzing Reagent (sold separately). In a pre-treatment step, whole blood is mixed with the Hemolyzing reagent in a 1:100 dilution

and the resultant hemolysate is used. Tetradecylmethyammonium bromide (TTB) in the hemolyzing reagent eliminated 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).

The HbA1c Calibrators are provided in 5 lyophilized levels which contain: Hemolystae (human and sheep), 0.9% tetradecyltrimethylammonium bromide

J. Substantial Equivalence Information:

1. Predicate device name(s):

Synchron Systems Hemoglobin A1c reagent

2. Predicate 510(k) number(s):

k010748

3. Comparison with predicate:

Similarities and Differences:Reagent		
Item	Candidate Device HbA1c (Hemoglobin A1c) reagent (k120199)	Predicate Device Synchron Systems Hemoglobin A1c reagent (k010748)
Intended Use/Indications for 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 HbA1c reagent kit, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s). SYNCHRON ®Systems HbA1c Calibrators and SYNCHRON® Systems Hemolyzing Reagent, is intended for the quantitative determination of hemoglobin A1c concentration as a percentage of total hemoglobin in human whole blood
Technology	Colorimetric	Same
Analytical	4-15% HbA1c (NGSP)/20-140	2-20% HbA1c

Similarities and Differences:Reagent		
Item	Candidate Device HbA1c (Hemoglobin A1c) reagent (k120199)	Predicate Device Synchron Systems Hemoglobin A1c reagent (k010748)
Measuring Range	mmol/mol (IFCC)	
Methodology	Turbidimetric Inhibition	Same
Sample Types	K_2-EDTA, K^3-EDTA, Li-Heparin or Na-Heparin (freshly drawn blood treated with EDTA is preferred)	Whole blood EDTA or heparin (freshly drawn blood treated with EDTA is preferred)

Similarities and Differences: Calibrator		
Item	Candidate Device (k120199)	Predicate Device (k010748)
Intended Use/Indications for Use	The HbA1c Calibrators are an in vitro diagnostic product for the calibration of the hemoglobin A1c (HbA1c) method on the AU clinical chemistry systems.	For calibration of HbA1c on the Synchron Systems analyzers when used in conjunction with the HbA1c reagent on the UniCel® DxC 600/800 System(s).
Calibrator base matrix	Hemolysate (human and sheep)	Same
Calibrator Format and Levels	Lyophilized 5 levels THb=two point calibration HbA1c=multi point calibration	Lyophilized 5 levels THb=single point HbA1c=multi point calibration

K. Standard/Guidance Document Referenced (if applicable):

CLSI C28-A3, Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition

CLSI-EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition (Interim Revision)

CLSI-EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

CLSI-EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

Draft Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs

L. Test Principle:

Total Hemoglobin Reagent is used to measure total hemoglobin 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were performed according to CLSI EP05-A2. Within-run and Total precision were evaluated by testing a commercial low and high control treated with hemolyzing reagent. A medium control pool was prepared by mixing the low and high pool and treated with hemolyzing reagent. Each sample pool was assayed 2 replicates per run, 2 runs per day, for 20 days (n=80) on the Beckman AU480, AU680 and AU2700 analyzers.

An additional precision study was performed according to CLSI-EP05-A2. Within-run and Total precision were evaluated using three natural patient EDTA blood sample pools. A low sample (4-5% HbA1c) a medium sample (~6.5% HbA1c) a high sample (~8.0% HbA1c) and a very high sample pool (12-14% HbA1c) were analyzed in triplicate, once a day over 5 days (n=15).

The results of both precision studies are shown below:

AU480 analyzer

Sample	N	Mean (%A1c)	Within Run		Total Imprecision	
			SD	%CV	SD	%CV
Low Control Pool	80	5.38	0.05	1.02	0.11	2.07
Medium Control Pool	80	7.44	0.05	0.70	0.10	1.36

High Control Pool	80	9.53	0.05	0.53	0.11	1.17
Low Blood Pool (4-5%)	15	4.92	0.05	1.0	0.08	1.6
Medium Blood Pool (6.5%)	15	6.80	0.06	0.8	0.06	0.8
High Blood Pool (~8%)	15	8.40	0.03	0.3	0.08	0.9
High Blood Pool (12-14%)	15	12.47	0.05	0.4	0.08	0.6

AU680 Analyzer

Sample	N	Mean (%A1c)	Within Run		Total Imprecision	
			SD	%CV	SD	%CV
Low Control Pool	80	5.35	0.06	1.09	0.07	1.34
Medium Control Pool	80	7.43	0.06	0.87	0.10	1.33
High Control Pool	80	9.66	0.07	0.75	0.09	0.93
Low Blood Pool (4-5%)	15	4.94	0.09	1.7	0.09	1.7
Medium Blood Pool (6.5%)	15	6.87	0.05	0.7	0.06	0.9
High Blood Pool (~8%)	15	8.47	0.05	0.6	0.07	0.8
High Blood Pool (12-14%)	15	12.92	0.05	0.4	0.13	1.0

AU2700 Analyzer

Sample	N	Mean (%A1c)	Within Run		Total Imprecision	
			SD	%CV	SD	%CV
Low Control Pool	80	5.30	0.08	1.44	0.11	2.07
Medium Control Pool	80	7.39	0.08	1.03	0.14	1.84
High Control Pool	80	9.39	0.10	1.03	0.16	1.68
Low Blood Pool (4-5%)	15	4.86	0.12	2.4	0.14	2.8
Medium Blood Pool (6.5%)	15	6.81	0.12	1.8	0.16	2.4
High Blood Pool (~8%)	15	8.40	0.09	1.1	0.09	1.1
High Blood Pool (12-14%)	15	12.47	0.21	1.7	0.24	1.9

b. *Linearity/assay reportable range:*

The claimed linearity range is 4-15% for HbA1c

Linearity was evaluated according to CLSI EP-06A. A series of eleven analyte concentrations, covering the measuring range of the assay, were prepared by interdilution of a high (19.6) and low (3.6) %HbA1c. Each dilution was assayed in quadruplicate and the mean analytical results were plotted versus the relative analyte concentration. Data was analyzed using 1st, 2nd, and 3rd order least square regressions according to CLSI Protocol EP6-A. Sponsor chose the 3rd order because it was the best fit. The percent bias between the 1st order and the 2nd order was <10%.

1st order $y=0.22x-2.09$

2nd order $y=0.00x^2+0.20x-2.32$

3rd order $y=9.61x^3-0.00x^2+0.16x+2.67$

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The Beckman Coulter HbA1c Test System assay standardization is traceable to the IFCC reference calibrators. The Beckman Coulter HbA1c Test System assay is certified with the National Glycohemoglobin Standardization Program (NGSP), with an expiration date of September 1, 2013. The NGSP certification expires one year from the certification date and needs to be renewed annually. See NGSP website for current certification at http://www.ngsp.org

Two different units of measure are provided to the customers: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

The HbA1c Calibrators are stable when unopened and stored at 2-8˚C up to the stated expiration date printed on the bottle. Reconstituted calibrators are stable for 8 hours when stored at 15-25˚C or 30 hours when stored at 2-8˚C until the expiration date is reached. Calibrators are stable for 30 days when reconstituted and stored at -20˚C.]Protocols and acceptance criteria were reviewed and found acceptable to support the claimed conditions.

Calibrator values are assigned using multiple analyzers and multiple runs over several days. The data generated provides set points for use on the AU480, AU680, and AU2700 platforms. Once the calibration set points are established on the analyzers; principal samples (IFCC calibrators, IFCC controls, freshly prepared test calibrators, NGSP traceable samples to cover the NGSP range, and HbA1c/Thb linearity sets) are run in triplicate and must meet the sponsor's pre-determined acceptance criteria.

Controls- The sponsor recommends any commercially available control for HbA1c. Alternatively, the user may use controls previously cleared in *k043070*

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined by assaying an analyte free sample (blank) and five low level HbA1c samples according to CLSI guideline EP17A. Each sample was assayed once a day for 12 days on the Beckman AU480, AU680 and AU2700 analyzers. The detection limits are summarized in the table below:

HbA1c

Platform/Method	LoB (%A1c)	LoD (%A1c)
Beckman AU480, AU680 and AU2700 analyzer	2.2	2.2

The assay has a reportable range of 4.0-15.0% for HbA1c on the Beckman AU480, AU680 and AU2700 analyzer.

e. Analytical specificity:

i.) Interference studies were performed to assess common or known substances that could interfere with the AU® Systems HbA1c (Hemoglobin A1c) Test System. The interfering substances were evaluated using K^2 EDTA whole blood samples. Sample pools were tested at two HbA1c concentrations (~5% and ~10%). Each sample was tested in quadruplicate to give a total of four replicates per sample on the Beckman AU460, AU680 and the AU2700 Clinical Systems using the AU CLINICAL SYSTEMS HbA1c assay. The percent difference of the samples with and without the potential interfering substance was calculated. The sponsor's acceptance criteria is analyte recovery should not vary from the base recovery by more than 6%. No significant interference was defined as the % recovery of $\leq \pm$ 6% for Unconjugated Bilirubin, Rheumatoid Factor and Ascorbic Acid. No significant interference was defined as the % recovery of $\leq \pm$ 7% for Triglycerides.

The sponsor claimed that there was no significant interference by the following interfering substances:

- Unconjugated bilirubin up to 30 mg/dL
- Triglycerides (Intralipid) up to 400 mg/dL
- Rheumatoid Factor up to 1000 IU/mL
- Ascorbic Acid 50 mg/dL
- Labile glycated hemoglobin up to 2000 mg/dL

ii.) An interference study was performed to assess the affect of labile A1c with the AU® Systems HbA1c (Hemoglobin A1c) Test System. Two levels of EDTA whole blood samples (~5% and ~10% A1c) were used and each pool was split into two aliquots. One aliquot was used as the control sample while the other

aliquot was supplemented with glucose to a glucose concentration of 2000 mg/dL. The aliquots were incubated for 1 hour at 37 degrees Celsius and then tested on the Beckman AU480, AU680 and AU2700 Clinical Systems using the. AU® Systems HbA1c (Hemoglobin A1c) Test System. The percent difference of the samples with and without the potential interfering substance was calculated. The sponsor's acceptance criteria is analyte recovery should not vary from the base recovery by more than 10%. No significant interference was defined as % recovery of ≤ ± 10%.

The sponsor claimed that there was no significant interference with labile A1c concentrations up to 2000mg/dL

iii.) To study the effect of carbamylated hemoglobin, two EDTA whole blood patient samples with A1c concentration of ~5% and ~10% were split into two aliquots. Sodium cyanate (10.5 mg/ml in stock solution) was added to one aliquot of each concentration. Samples were incubated at 37 degrees Celsius for >1 hour. Samples were tested using the AU® Systems HbA1c (Hemoglobin A1c) Test System. The sponsor's acceptance criterion is ≤10% bias between the tested and the control samples.

The sponsor concludes that carbamylated A1c does not interfere with the AU® Systems HbA1c (Hemoglobin A1c) Test System .

iv.) An interference study was performed to assess the effect of acetylsalicylic acid on the AU® Systems HbA1c (Hemoglobin A1c) Test System. Two EDTA whole blood patient samples with A1c concentration of ~5% and ~10% were split into two aliquots. One aliquot of each concentration was supplemented by adding acetylsalicylic acid (10.1 mg/ml in stock solution). The samples were tested using the AU CLINICAL SYSTEMS HbA1c assay. The sponsor's acceptance criterion is ≤10% bias between the tested and the control samples.

The sponsor concludes that acetylsalicylic acid does not interfere with the AU® Systems HbA1c (Hemoglobin A1c) Test System.

v.) A hemoglobin variant study was performed using commercial samples known to contain Hemoglobin variants C, D, E, S, F. These variant samples were tested on the AU 680 Clinical System using the AU® Systems HbA1c (Hemoglobin A1c) Test System. All variants tested showed <10% bias at ~5% and ~7% for HbC, ~5%, ~7% and ~9% for HbD, ~6% and ~9 HbE, ~5%, ~7% and ~10% for HbS and ~5 and ~7% for HbF.

The labeling states "Samples containing >10% HbF may result in lower than expected HbA1c results."

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed using the AU®Systems HbA1c (Hemoglobin) Test system on the AU480, AU680 and the AU2700 analyzers versus the Synchron Systems Hemoglobin A1c (HbA1c) Reagent on the Beckman DXC800 analyzer. The study was completed using 130 whole blood donor samples. Each sample was analyzed in singlicate using the candidate device and the predicate device. Sample range tested was 4.9- 14.2% HbA1c. The linear regression correlation is summarized below:

Analyzer	Sample Range	n	R	Slope	Intercept
AU480	4.9-14.2% HbA1c	130	0.9930	0.913	0.322
AU680	4.9-14.2% HbA1c	130	0.9941	0.901	0.3140
AU2700	4.9-14.2% HbA1c	130	0.9927	0.905	0.3276

Another method comparison study was performed using the AU®Systems HbA1c (Hemoglobin) Test system on the AU480, AU680 and the AU2700 analyzers versus the Olympus Hemoglobin A1c (HbA1c) Reagent (k031380) on the same analyzers. The study was completed using 116 whole blood donor samples. Each sample was analyzed in singlicate using the candidate device and the predicate device. Sample range tested was 4.6- 12.0% HbA1c. The linear regression correlation is summarized below:

Analyzer	Sample Range	n	R	Slope	Intercept
AU480	4.6-12.0% HbA1c	116	0.9962	1.028	-0.2211
AU680	4.6-12.0% HbA1c	116	0.9968	1.036	-0.3821
AU2700	4.6-12.0% HbA1c	116	0.9957	1.024	-0.4150

b. Matrix comparison:

A matrix comparison study was performed using K_3 EDTA, Lithium Heparin, and Na-Heparin. K_2EDTA was used as the reference anticoagulant. 52 total samples were analyzed for HbA1c. Each single set of samples were analyzed on the Beckman AU680 Clinical Chemistry analyzer. Samples ranged from 4.8% -14.6% HbA1c. The results using Deming regression analysis are as follows:

Anticoagulant	n	Deming Regression Analysis
K_3 EDTA	52	Y=0.993x+0.029; r=0.999
Lithium Heparin	52	Y=0.986x + 0.055; r=0.999
Sodium Heparin	52	Y=0.993x+0.031; r=0.999

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

   Not applicable

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

   The expected normal HbA1c range in adults is 4.0-6.0% (NGSP); 20-42 mmol/mol (IFCC units) [1,2,3]

   [1]Panteghini M, John WG. Implementation of Hemoglobin A1c results traceable to the IFCC reference system: the way forward. Clin Chem Lab Med 2007; 45(8):942-944.

   [2]Wu, A.,ed., Tietz clinical guide to Laboratory Tests, 4th edition

   [3]McPherson, R.A, Pincus, M.R., Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd Edition

   The sponsor recommends in the labeling that each clinical laboratory should establish its own reference range/expected values as dictated by good laboratory practices.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.