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

A. 510(k) Number:

k170623

B. Purpose for Submission:

Modification of previously cleared systems with a new data acquisition method

C. Measurand:

Whole Blood Glycosylated Hemoglobin (HbA1c)

D. Type of Test:

Quantitative, Antibody-coated latex agglutination inhibition

E. Applicant:

Alfa Wassermann Diagnostic Technologies, LLC.

F. Proprietary and Established Names:

ACE Hemoglobin A1c (HbA1c) Reagent

G. Regulatory Information:

Classification Name	Regulation Section	Device Class	Product Code	Panel
Glycosylated Hemoglobin Assay	21 CFR 864.7470	II	LCP	Hematology (81)

H. Intended Use:

1. Intended use(s):

   See Indication(s) for use below.

2. Indication(s) for use:

   ACE Hemoglobin A1c (HbA1c) Reagent is intended for the quantitative determination of percent hemoglobin A1c in venous whole blood collected in K2-EDTA tubes using the ACE Alera and ACE Axcel Clinical Chemistry Systems. This test is intended for use in clinical laboratories and physician office laboratories to monitor long term blood glucose control in individuals with diabetes mellitus. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use only.

   For in vitro diagnostic use only.

   This test is not intended for screening or diagnosis of diabetes mellitus.

   This test is not intended for use in neonates.

   This test is not useful in judging day-to-day glucose control and should not be used to replace daily home testing of urine and blood glucose.

   Diseases and other conditions that affect red blood cell survival (e.g., hemolytic anemia or other hemolytic diseases, pregnancy, significant or chronic blood loss), will cause a decrease in results for % Hemoglobin A1c and are not reliable.

   Hemoglobinopathies may interfere with glycated hemoglobin analysis. The results from both ACE Alera and ACE Axcel Clinical Chemistry Systems show that there is no significant interference for Hemoglobin D (≤ 36.3%) or Hemoglobin E (≤ 22.5%). High Hemoglobin F (> 10.1%) will result in lower than expected HbA1c values. High Hemoglobin C (> 14.0%) and high Hemoglobin S (> 17.1%) will result in higher than expected HbA1c values.

4. Special instrument requirements:

   ACE Alera and ACE Axcel Clinical Chemistry Systems

I. Device Description:

The ACE Hemoglobin A1c (HbA1c) Reagent kit contains the following:

- 2 x 25 mL Hemoglobin Denaturant: Porcine Pepsin, Buffer and Preservative
- 2 x 12 mL Total Hemoglobin Reagent: Sodium Hydroxide and Surfactant
- 1 x 11 mL Hemoglobin A1c Agglutinator Reagent: HbA1c Hapten Polymer, Bovine Serum Albumin, Buffer, Preservative and Surfactant
- 1 x 11 mL Hemoglobin A1c Antibody Reagent: HbA1c Antibody (mouse) Coupled Particles, Bovine Serum Albumin, Buffer, Preservative and Surfactant

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Hemoglobin A1c Reagent on DCA2000+ System

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

   k951361

3. Comparison with predicate:

Similarities/Differences Assay		
Item	New Device ACE Hemoglobin A1c (HbA1c) Reagent (k170623)	Predicate Hemoglobin A1c Reagent on DCA2000+ System (k951361)
Intended Use	Quantitative determination of percent hemoglobin A1c in venous whole blood. This test is intended to monitor long term blood glucose control in individuals with diabetes mellitus.	Same
Methodology	Antibody-coated latex agglutination inhibition	Same
Instrument Platforms	ACE Alera and ACE Axcel Clinical Chemistry Systems	DCA 2000+ Analyzer
Method Traceability or Standardization	National Glycohemoglobin Standardization Program (NGSP)	Same
Calibration	6 points for HbA1c, 1 point for total hemoglobin	Calibration card
Sample Type	Venous whole blood (K_2-EDTA)	Venous whole blood (EDTA, heparin, citrate and fluoride/oxalate), capillary whole blood
Sample Pretreatment	Manual sample pretreatment with hemoglobin denaturant	Automatic sample pretreatment
Testing Environment	Clinical laboratories and physician office laboratories	Same
Measuring range	2.7% - 13.0% HbA1c	2.5% - 14.0% HbA1c

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

CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline, Third Edition

CLSI EP06-A, Evaluation of the Linearity of Quantitative Analytical Measurement Procedure: A Statistical Approach, Approved Guideline

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

CLSI: EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples, Approved Guideline, Third Edition

CLSI EP15-A3, User Verification of Performance for Precision and Estimation of Bias; Approved Guideline, Third Edition

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline, Second Edition

L. Test Principle:

The ACE Hemoglobin A1c (HbA1c) Reagent assay requires a pretreatment step of the whole blood samples, which is done off-line. The red blood cells in the sample are lysed by the Hemoglobin Denaturant and the hemoglobin chains are hydrolyzed. For determination of HbA1c, a latex agglutination inhibition assay is used. HbA1c in the sample inhibits agglutination of the polymer agglutinator and the antibody-coated microparticles in the HbA1c Antibody Reagent. The increase in absorbance, monitored monochromatically at 692 nm, is inversely proportional to the HbA1c present in the sample. The HbA1c concentration is calculated using delta data acquisition method, instead of the previously cleared quadratic method, for the same ACE HbA1c Reagent on the ACE Alera and ACE Axcel Clinical Chemistry Systems. All hemoglobin derivatives in the sample are converted to alkaline hematin in order to determine total hemoglobin. The reaction produces a green colored solution, which is measured bichromatically at 573 nm/692 nm. The intensity of color produced is directly proportional to the total hemoglobin concentration in the sample. The concentrations of both HbA1c and total hemoglobin are measured, the ratio is calculated and the result reported as percent HbA1c.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

Internal Precision Study

Precision studies were performed according to CLSI EP05-A3 guideline. The study included four whole blood samples prepared from pooled K_2-EDTA venous specimens. Samples were analyzed in duplicate per run, two runs per day for 20 days on both ACE Alera and ACE Axcel Clinical Chemistry Systems. Results are shown below:

ACE Alera Clinical Chemistry Systems					
Sample	Mean (N=80)	Within-Run		Total	
		SD	%CV	SD	%CV
1	5.1	0.07	1.4	0.13	2.5
2	6.8	0.14	2.1	0.18	2.6
3	8.2	0.10	1.3	0.20	2.4
4	11.2	0.32	2.8	0.34	3.1
ACE Axcel Clinical Chemistry Systems					
Sample	Mean (N=80)	Within-Run		Total	
		SD	%CV	SD	%CV
1	5.0	0.08	1.6	0.12	2.4
2	6.8	0.14	2.0	0.17	2.6
3	8.2	0.13	1.6	0.21	2.6
4	11.1	0.27	2.4	0.32	2.9

External Precision Study

An external precision study using four K_2-EDTA venous whole blood samples was performed at three Physician's Office Laboratories for ACE Alera and ACE Axcel Clinical Chemistry Systems using one lot of reagent, respectively. Samples were tested in triplicates per day for 5 days on one instrument by one operator at each site. Results from each site are shown below:

ACE Alera Clinical Chemistry Systems						
Lab #	Sample	Mean (N=15)	Within-Run		Total	
			SD	%CV	SD	%CV
1	1	5.3	0.09	1.6	0.09	1.6
2		5.2	0.11	2.1	0.12	2.4
3		4.6	0.08	1.8	0.13	2.9
1	2	6.8	0.12	1.7	0.12	1.7
2		6.7	0.08	1.2	0.14	2.1
3		6.0	0.07	1.1	0.14	2.3
1	3	8.8	0.24	2.7	0.27	3.1
2		8.5	0.11	1.3	0.18	2.1
3		7.9	0.07	0.9	0.25	3.1
1	4	12.5	0.29	2.4	0.32	2.5
2		12.5	0.36	2.9	0.36	2.9
3		11.5	0.13	1.1	0.27	2.3

ACE Axcel Clinical Chemistry Systems						
Lab #	Sample	Mean (N=15)	Within-Run		Total	
			SD	%CV	SD	%CV
1	1	5.4	0.12	2.2	0.24	4.3
2		4.6	0.09	2.0	0.11	2.3
3		4.8	0.10	2.1	0.10	2.2
1	2	6.8	0.08	1.1	0.13	1.9
2		6.1	0.13	2.2	0.16	2.7
3		6.3	0.11	1.7	0.12	1.8
1	3	8.8	0.12	1.3	0.20	2.2
2		8.8	0.16	1.8	0.24	2.8
3		9.1	0.15	1.7	0.27	2.9
1	4	11.9	0.19	1.6	0.29	2.5
2		11.8	0.34	2.9	0.42	3.5
3		12.2	0.15	1.2	0.35	2.9

b. *Linearity/assay reportable range:*

Linearity was evaluated according to CLSI EP06-A. Specific dilutions of a high sample were prepared to make a total of 11 samples with concentrations covering the assay range (2.7, 3.7, 4.8, 5.8, 6.8, 7.8, 8.9, 9.9, 10.9, 11.9 and 13.0%). All samples were analyzed in triplicates on one ACE Alera and one ACE Axcel Clinical Chemistry Systems. The mean of these replicates was compared to the expected values and the results of linear regressions are summarized below:

ACE Alera Clinical Chemistry System: $y = 0.987x + 0.3$, $r^2 = 0.995$
ACE Axcel Clinical Chemistry System: $y = 0.954x + 0.3$, $r^2 = 0.994$

The study supports the sponsor's claimed linearity range of 2.7 - 13.0% HbA1c on both ACE Alera and ACE Axcel Clinical Chemistry Systems.

Acceptable total hemoglobin/hematocrit ranges for the HbA1c measurement was determined following CLSI EP06-A. Specific dilutions of a high hemoglobin sample were prepared to make a total of 11 samples. All samples were analyzed in four replicates on one ACE Alera and one ACE Axcel Clinical Chemistry Systems. The mean of these replicates was compared to the expected values and the results of linear regressions are summarized below:

ACE Alera Clinical Chemistry System: $y = 1.006x + 0.10$, $r^2 = 0.998$
ACE Axcel Clinical Chemistry System: $y = 0.997x + 0.20$, $r^2 = 0.996$

The study supports the sponsor's claimed reportable range of 1.4 to 21.8 g/dL for total hemoglobin on both ACE Alera and ACE Axcel Clinical Chemistry Systems.

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

Traceability:
The ACE Hemoglobin A1c (HbA1c) Reagent is certified by the National Glycohemoglobin Standardization Program (NGSP). The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org.

d. *Detection limit:*

A Limit of Quantitation (LoQ) study was performed according to the CLSI EP17-A2 guideline. Five whole blood samples with low analyte concentrations were tested over 5 days with 8 replicates per day using one lot of the ACE Hemoglobin A1c (HbA1c) Reagent on both ACE Alera and ACE Axcel Clinical Chemistry Systems. LoQ was defined as the lowest concentration at which %CV was < 20% and was determined to be 2.5%.

e. *Analytical specificity:*

Endogenous substances:
Interference studies were performed according to recommendations in the CLSI EP07-A2 guideline. Various concentrations of potential endogenous interferents were spiked into two levels of whole blood samples (4.9-5.5% HbA1c and 6.9-7.8% HbA1c). All samples were tested in triplicate on both ACE Alera and ACE Axcel Clinical Chemistry Systems. The sponsor defined non-significant interference as ≤ 10% bias in recovery for spiked samples when compared to control samples. The highest endogenous substance concentrations tested that demonstrated no interference are shown below:

Substance	**Highest Concentration tested at which no interference was observed**
Bilirubin	53 mg/dL
Triglycerides	1100 mg/dL
Ascorbic Acid	6 mg/dL
Acetaldehyde	100 mg/dL
Sodium Fluoride	1200 mg/dL

Hemoglobin variant interference:
A hemoglobin variant study was performed using 15 K2-EDTA venous whole blood samples (4.7 – 12.0% HbA1c) containing known levels of hemoglobin variants C, D, E, F and S. Three to five samples per hemoglobin variant were tested in four replicates on both ACE Alera and ACE Axcel Clinical Chemistry Systems for %HbA1c results. Percent recovery was calculated by comparing to the results obtained on comparator methods free of interference with the respective hemoglobin variant (Trinity Biotech Premier Hb9210, k112015, for HbC, HbD, HbS, Primus

Ultra 2 Boronate Affinity HPLC, k891235, for HbE and Tosoh G8, k071132 for HbF). Non-significant interference was defined as recovery within 100 ± 10%.

The testing results indicate that there is no significant interference from Hemoglobin D (≤ 36.3%) or Hemoglobin E (≤ 22.5%). Hemoglobin F (> 10.1%) will result in lower than expected HbA1c results, while Hemoglobin C (> 14.0%) and Hemoglobin S (> 17.1%) will result in higher than expected HbA1c results.

The labeling contains the following limitation statements for Hb variant interference:

"Hemoglobinopathies may interfere with glycated hemoglobin analysis. The results from both ACE Alera and ACE Axcel Clinical Chemistry Systems show that there is no significant interference for Hemoglobin D (≤ 36.3%) or Hemoglobin E (≤ 22.5%). High Hemoglobin F (> 10.1%) will result in lower than expected HbA1c values. High Hemoglobin C (> 14.0%) and high Hemoglobin S (> 17.1%) will result in higher than expected HbA1c values."

Labile A1c interference:
Two HbA1c levels of whole blood samples were spiked with glucose to contain 360, 720, 1080 and 1440 mg/dL glucose and then incubated for four hours at 37°C to facilitate the formation of labile A1c. The samples were tested in four replicates on both ACE Alera and ACE Axcel Clinical Chemistry Systems. The sponsor defined non-significant interference as within 10% difference between samples containing glucose and the control sample. The results support the sponsor's claim that labile A1c showed no significant interference at 1440 mg/dL glucose level.

Carbamylated hemoglobin interference:
Two HbA1c levels of whole blood samples were spiked with Sodium Cyanate to contain 500, 1000, 1500 and 2000 mg/dL of sodium cyanate. The samples were tested in four replicates on both ACE Alera and ACE Axcel Clinical Chemistry Systems. The sponsor defined non-significant interference as within 10% difference between samples containing Sodium Cyanate and the control sample. The results support the sponsor's claim that Carbamylated Hb showed no significant interference at 2000 mg/dL Sodium Cyanate level.

Acetylated hemoglobin interference:
Two HbA1c levels of whole blood samples were spiked with Acetylsalicylic Acid to contain 500, 1000, 1500 and 2000 mg/dL of Acetylsalicylic Acid. The samples were tested in four replicates on both ACE Alera and ACE Axcel Clinical Chemistry Systems. The sponsor defined non-significant interference as within 10% difference between samples containing Acetylsalicylic Acid and the control sample. The results support the sponsor's claim that acetylated hemoglobin showed no significant interference at 2000 mg/dL Acetylsalicylic Acid level.

HbA0 and HbA1a+b interference:
Two HbA1c levels of whole blood samples were spiked with HbA1a+b to contain 25,

50, 75 and 100 mg/dL of HbA1a+b. The samples were tested in four replicates on both ACE Alera and ACE Axcel Clinical Chemistry Systems. The sponsor defined non-significant interference as within 10% difference between samples containing HbA1a+b and the control sample. The results support the sponsor's claim that HbA1a+b fraction showed no significant interference at 100 mg/dL.

Two HbA1c levels of whole blood samples were spiked with HbA0 to contain 575, 1150, 1725 and 2300 mg/dL of HbA0. The samples were tested in four replicates on both ACE Alera and ACE Axcel Clinical Chemistry Systems. The sponsor defined non-significant interference as within 10% difference between samples containing HbA0 and the control sample. The results support the sponsor's claim that HbA0 fraction showed no significant interference at 1725 mg/dL.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was conducted internally by testing 101 and 102 K_2-EDTA venous whole blood patient samples in singlicate on one ACE Alera and one ACE Axcel Clinical Chemistry System, respectively, against the predicate device. Results of the linear regression are shown below:

Device	ACE Alera vs. DCA 2000+	ACE Axcel vs. DCA 2000+
N	101	102
Range (x-method)	3.2% - 12.8% HbA1c	2.5% - 12.8% HbA1c
Regression Equation	y=0.979x+0.05	y=0.983x-0.03
Correlation Coefficient	0.9839	0.9832
Confidence Interval – slope	0.944 to 1.015	0.948 to 1.019
Confidence Interval - intercept	-0.21 to 0.31	-0.29 to 0.24

External method comparison studies were conducted at 3 Physician Office Laboratory (POL) sites for ACE Alera Clinical Chemistry System and 3 other POL sites for ACE Axcel Clinical Chemistry System. A total of 50-52 K_2-EDTA venous whole blood samples with ≤ 10% altered samples were tested on ACE Alera or ACE Axcel Clinical Chemistry System in singleton at each POL site with one lot of the ACE HbA1c reagent and compared to the predicate method. Deming regression analysis results are shown below:

ACE Alera

Site #	N	Range (x-method) %HbA1c	Regression Equation	Correlation Coefficient	Confidence Interval – slope	Confidence Interval - intercept
1	50	3.2 – 13.4	y=0.967x+0.34	0.9892	0.925 to 1.008	0.04 to 0.65
2	50	3.1 – 12.9	y=0.984x-0.02	0.9945	0.955 to 1.014	-0.23 to 0.18
3	50	3.2 – 12.4	y=0.981x-0.09	0.9939	0.950 to 1.013	-0.30 to 0.12

ACE Axcel

Site #	N	Range (x-method) %HbA1c	Regression Equation	Correlation Coefficient	Confidence Interval – slope	Confidence Interval - intercept
4	52	3.2 – 12.9	y=1.000x-0.28	0.9885	0.957 to 1.043	-0.59 to 0.03
5	50	3.4 – 12.8	y=0.993x-0.12	0.9932	0.960 to 1.027	-0.37 to 0.13
6	50	3.5 – 12.9	y=0.980x+0.02	0.9960	0.955 to 1.006	-0.16 to 0.20

b. Matrix comparison:

Not applicable. The ACE Hemoglobin A1c (HbA1c) Reagent is for use with K_2-EDTA venous whole blood samples only.

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:

Glycemic recommendation for many non-pregnant adults with diabetes:	
A1c	< 7.0%*

*More or less stringent glycemic goals may be appropriate for individual patients. Goals should be individualized based on duration of diabetes, age/life expectancy, comorbid conditions, known CVD or advanced microvascular complications, hypoglycemia unawareness, and individual patient considerations.

Source: American Diabetes Association. Glycemic Targets: Standards of Medical Care in Diabetes - 2018. Diabetes Care 2018 Jan; 41 (Supplement 1): S55-S64.

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.