

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

A. 510(k) Number:

k091711

B. Purpose for Submission:

New device

C. Measurand:

Whole blood hemoglobin A1c (HbA1c)

D. Type of Test:

Quantitative – colorimetric

E. Applicant:

Alfa Wassermann Diagnostic Technology, Inc.

F. Proprietary and Established Names:

S-Test % Hemoglobin A1c (HbA1c)

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LCP – Assay, Glycosylated Hemoglobin	Class II	21 CFR§ 864.7470	81

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The S-Test Hemoglobin A1c Reagent is intended for the quantitative determination of percent Hemoglobin A1c concentration in EDTA whole blood using the S40 Clinical Analyzer. Measurement of glycosylated hemoglobin is used for monitoring the long term glycemic control of individuals with diabetes. This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

S40 Clinical Analyzer

I. Device Description:

The S-Test Hemoglobin A1c reagent cartridges used with the S40 Clinical Analyzer are intended for quantitative *in vitro* diagnostic determination of % HbA1c in EDTA whole blood. The assay uses two bi-reagent single-use cartridges, which are plastic containers consisting of liquid stable reagents and reaction cavities, together with 2-D bar code labels. The two bi-reagent cartridges consist of a pre-treatment cartridge containing protease and N-ethylmaleimide and a measuring cartridge containing N-(carboxymethylaminocarbonyl) 4-4'-bis (dimethylamino) diphenylamine sodium salt, peroxidase and fructosyl peptide oxidase (FPOX). The bar codes contain all chemistry parameters, calibration factors, and other information.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Siemens Medical Systems Diagnostics DCA Vantage Test System for Hemoglobin A1c

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

k071466

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Sample type	EDTA whole blood	Same
Reaction type	Endpoint	Same

Differences		
Item	Device	Predicate
Indications for Use	intended for the quantitative determination of percent Hemoglobin A1c concentration in EDTA whole blood using the S40 Clinical Analyzer. Measurement of Hemoglobin A1c is used for monitoring the long term glycemic control of individuals with diabetes. This test is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.	designed to quantitatively measure the percent Hemoglobin A1c in blood. The measurement of hemoglobin A1c concentration is recommended for monitoring the longterm glycemic control of persons with diabetes.
Instrument	S40 Clinical Analyzer	DCA Analyzers
Measuring Range	3.8 to 14.0 % HbA1c	2.5 to 14.0 % HbA1c

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (2004)

CLSI EP10-A: Preliminary Evaluation of Quantitative Clinical Laboratory Methods; Approved Guideline –Second Edition (2002)

CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach: Approved Guideline (2003)

CLSI EP7-A: Interference Testing in Clinical Chemistry; Approved Guideline (2002)

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004)

CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (2002)

L. Test Principle:

In the first reaction, a glycosylated dipeptide is cleaved from the β -chain N-terminus of HbA1c by a protease, and the level of total hemoglobin (Hb) is determined using a bi-chromatic absorbance reading at 600/800 nm. In the second reaction, the glycosylated dipeptide is treated with fructosyl peptide oxidase (FPOX) to yield hydrogen peroxide and then, in the presence of peroxidase, (POD), the resulting

hydrogen peroxide is reacted with N-(carboxymethylaminocarbonyl) 4-4'-bis (dimethylamino) diphenylamine sodium salt (color coupler) to form a colored product. The level of HbA1C is determined by measuring the absorbance of the colored product bichromatically at 700/800 nm. Percentage (%) of HbA1c is calculated from the measured levels of total Hb and HbA1c.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Studies were carried out consistent with CLSI EP5-A2. Precision was determined on the S40 Clinical Analyzer at three % HbA1c levels using commercially available control material containing intact red blood cells. The three levels were assayed 2 times per run, 2 runs per day, for a total of 20 days. The mean, standard deviations, and % coefficients of variation (CV) were calculated for each sample.

<u>Sample 1</u> Mean = 4.6 % HbA1c	Within Run	Between Run	Between Day	Total
Standard deviation	0.05	0.00	0.02	0.06
Coefficient of Variation	1.1%	0.0%	0.4%	1.2%

<u>Sample 2</u> Mean = 6.7% HbA1c	Within Run	Between Run	Between Day	Total
Standard deviation	0.07	0.03	0.07	0.10
Coefficient of Variation	1.1%	0.4%	1.0%	1.5%

<u>Sample 3</u> Mean = 10.9% HbA1c	Within Run	Between Run	Between Day	Total
Standard deviation	0.10	0.03	0.08	0.13
Coefficient of Variation	0.9%	0.3%	0.7%	1.2%

Further precision studies were conducted at three separate physician's office laboratory (POL) sites using the S40 analyzer. One operator at each site performed the testing. Studies were carried out in accordance with CLSI EP 10-A3 testing commercially available control material containing intact red blood cells at three levels. The samples were run three times a day for five days using one instrument. The results are presented in the table below:

Lab	Sample	Mean	%CV or SD (unit)	
			Within-Run	Total
POL 1	1	4.5 %	SD 0.08	SD 0.08
			1.8%	1.8%
POL 2	1	4.6 %	SD 0.06	SD 0.06
			1.3%	1.3%
POL 3	1	4.5 %	SD 0.04	SD 0.04
			0.9%	1.0%
POL 1	2	6.6 %	SD 0.08	SD 0.08
			1.2%	1.2%
POL 2	2	6.6 %	SD 0.04	SD 0.08
			0.6 %	1.3%
POL 3	2	6.5 %	SD 0.08	SD 0.08
			1.2 %	1.2 %
POL 1	3	10.8 %	SD 0.16	SD 0.16
			1.5 %	1.5 %
POL 2	3	11.0 %	SD 0.13	SD 0.14
			1.2 %	1.2 %
POL 3	3	10.9 %	SD 0.05	SD 0.13
			0.5 %	1.2 %

Three EDTA whole blood samples (with normal, intermediate, and elevated levels) were tested for HbA1c on three S40 Clinical Analyzers (one at each lab) four times per run, one run per day, for a total of five days. Different sets of samples were run at each site. These studies were conducted at three different POL sites. The results are presented in the table below:

Lab	Sample	Mean	SD (%HbA1c) and CV (%)	
			Within Run	Total
POL 1	1	4.7	SD 0.06	SD 0.10
			1.3%	2.1%
POL 2	1	5.1	SD 0.06	SD 0.06
			1.2%	1.2%

POL 3	1	5.0	SD 0.08	SD 0.08
			1.5%	1.5%
POL 1	2	8.2	SD 0.08	SD 0.08
			1.0%	1.0%
POL 2	2	7.7	SD 0.06	SD 0.07
			0.8%	1.0%
POL 3	2	7.7	SD 0.05	SD 0.05
			0.7%	0.7%
POL 1	3	11.7	SD 0.12	SD 0.15
			1.0%	1.3%
POL 2	3	11.2	SD 0.07	SD 0.08
			0.6%	0.7%
POL 3	3	11.3	SD 0.06	SD 0.36
			0.5%	3.2%

b. Linearity/assay reportable range:

The reportable range is 3.8 to 14.0 % HbA1c.

Linearity across the assay range was confirmed by testing commercial linearity standards with known ratios of HbA1c at 8 levels. Each level was tested in replicates of four. The percent recoveries ranged from 98 to 104 %. The linear regression equation obtained for the study was $y = 1.001x - 0.025$, $r^2 = 0.9995$.

An additional linearity study was conducted using EDTA whole blood samples according to CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures. Two EDTA whole blood samples were mixed in six different proportions of HbA1c. Each level was tested in replicates of four. The percent recoveries ranged from 93.7 to 100.0 %. The linear regression equation obtained for the study was $y = 0.9658 + 0.11x$, $r^2 = 0.9895$. The samples tested for the studies had values ranging from 3.8 % HbA1c to 15.7 % HbA1c.

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

The S Test-HbA1c cartridges are factory calibrated and traceable to IFCC certified standard material. The S test-HbA1c cartridge assay has met the requirements of the National Glycohemoglobin Standardization Program (NGSP). The NGSP certification requires annual renewal. The current list of NGSP certified methods is available on the NGSP website at:

<http://www.ngsp.org/prog/index.html>

d. Detection limit:

The reportable range is 3.8 to 14.0 % HbA1c (see linearity section above).

e. *Analytical specificity:*

Interference studies were performed according to CLSI guideline EP7-A2 to determine the effects of bilirubin, triglyceride, ascorbic acid, labile HbA1c (glucose), acetaldehyde, acetylsalicylic acid, sodium cyanate, and hemoglobin variants (Hb C, Hb D-Los Angeles, HbE and HbS). Two whole blood samples with levels of HbA1c ranging from 5.2 to 11.0 % HbA1c were tested for each interferent.

Bilirubin: No significant interference ($\leq 10\%$) up to 40 mg/dL

Triglyceride: No significant interference up to 750 mg/dL

Ascorbic acid: No significant interference up to 100 mg/dL

Labile HbA1c (glucose): No significant interference up to 1000 mg/dL

Acetaldehyde hemoglobin: No significant interference up to 100 mg/dL

Acetylsalicylic acid: No significant interference up to 100 mg/dL

Sodium cyanate: No significant interference up to 100 mg/dL

Hemoglobin variants (Hb C, Hb D-Los Angeles, HbE and HbS): 16 % negative interference as stated in labeling

The labeling contains a statement that triglyceride concentrations greater than 750 mg/dL may cause interference.

Studies demonstrated that the HbA1c assay yields accurate results over the range of 6 to 23 g/dL total Hb. For samples with total Hb results outside this range, the results will be flagged with a "CALC" error. Samples with normal and elevated HbA1c values and total hemoglobin concentrations ranging from 4.0 to 25.3 were tested and compared to control samples with known HbA1c values. Recoveries for samples with total Hb between 6 and 23 g/dL ranged from 99 to 100%.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A series of 110 EDTA whole blood specimens with HbA1c values ranging

from 4.0 to 13.4 % were assayed in singlicate on the S40 Clinical Analyzer using S-Test HbA1c Reagent and on the comparative method, Siemens DCA. Least-squares regression analysis (Deming) yielded the following results:

Regression Equation	$y = 1.025x - 0.23$
Correlation Coefficient	0.9821
Std. Error Est.	0.39
Confidence Interval Slope	0.988 to 1.062
Confidence Interval Intercept	-0.48 to 0.02

Point of Care in Physician’s Office Laboratories

Performance for the S-Test HbA1c assay was evaluated at three Physician Office Laboratories and with a total of three operators. Operators ran unaltered clinical EDTA whole blood samples with values ranging from 4.5 to 13.4 %. The correlation study between the device and the predicate for serum yielded the following results.

POL Lab	1	2	3
N	56	50	47
Range (mg/L)	4.5 – 12.4 %	4.5 – 13.4 %	4.5 – 13.2 %
Regression equation	$y = 0.993x - 0.45$	$y = 0.987x - 0.37$	$y = 1.017x - 0.20$
Correlation coefficient	0.9955	0.9943	0.9881
Standard error	0.18	0.26	0.31
Confidence interval slope	0.967 to 1.018	0.957 to 1.018	0.970 to 1.065
Confidence interval intercept	-0.64 to -0.27	-0.63 to -0.11	-0.55 to 0.16

b. Matrix comparison:

Not applicable since EDTA whole blood is the only sample type indicated.

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:

Using the S-test HbA1c method, blood samples from 133 apparently healthy individuals (fasting blood glucose <120 mg/dL) were assayed. The expected values range for the S-test HbA1c reagent was determined as the central 95 % of the results and ranged from 4.5 to 5.8 %. The American Diabetes Association (ADA) expected values range is 4.0 to 6.0 % for people without diabetes, according to the American Diabetes Association, Standards of Medical Care in Diabetes -2009, Diabetes Care, 32:Suppl 1 (2009). Both the S-test expected vales and ADA expected values are included in the labeling.

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.