A. 510(k) Number:

k091333

B. Purpose for Submission:

New device

C. Measurand:

Low Density Lipoprotein Cholesterol (LDL-C)

D. Type of Test:

LDL-C reagent cartridges, used with the S40 Clinical Analyzer, intended for the quantitative determination of LDL-C based on an enzymatic photometric assay.

E. Applicant:

Alfa Wassermann, Inc.

F. Proprietary and Established Names:

S-Test LDL Cholesterol (LDL)

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1475 – Lipoprotein Test System

2. Classification:

   Class I, meets the limitations to the exemption (21 CFR 862.9(c)(4))

3. Product code:

   MRR – System, Test, Low Density Lipoprotein

4. Panel:

Clinical Chemistry

H. Intended Use:

1. Intended use(s):

   Refer to indications for use below.

2. Indication(s) for use:

   The S-Test Low Density Lipoprotein Cholesterol Reagent is intended for the quantitative determination of LDL concentration in serum or heparin plasma using the S40 Clinical Analyzer. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, various liver and renal diseases. 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.

   For *in vitro* diagnostic use only.

4. Special instrument requirements:

   S40 Clinical Analyzer

I. Device Description:

The S-Test Low Density Lipoprotein (LDL) cholesterol reagent cartridge, used with the S40 Clinical Analyzer, is a single-use bi-reagent plastic cartridge consisting of liquid stable reagents and a reaction cavity, together with a bar code label. The bar code contains all chemistry parameters, calibration factors, and other production-related information. The bi-reagent cartridge contains the following materials: 4-Aminoantipyrine (0.01%), cholesterol esterase (< 2.5 U/mL), cholesterol oxidase (1.2 IU/mL), peroxidase (< 1.3 ppg U/mL), surfactant 1 and 2, DSBmT (0.04%), and Good's Buffer (pH 6.3).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ACE Low Density Lipoprotein Cholesterol Reagent

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

k991733

3. <u>Comparison with predicate:</u>

Comparison Table		
Item	Device (k091333)	Predicate (k991733)
Indications for Use	The S-Test Low Density Lipoprotein Cholesterol Reagent is intended for the quantitative determination of LDL concentration in serum or heparin plasma using the S40 Clinical Analyzer. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, various liver and renal diseases, and for the assessment of the risk of developing cardiovascular disease. This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.	ACE LDL-C Reagent is intended for the quantitative determination of low density lipoprotein cholesterol (LDL-C) in serum using the ACE and NExCT clinical chemistry systems. For *in vitro* diagnostic use only.
Measurand	LDL Cholesterol	LDL Cholesterol
Dimensions	Two cartridge reservoirs to deliver 210 μL and then 70 μL of reagent to reaction cuvette	Bottles with total volumes of 10 and 30 mL containing reagents.
Instrument	S40 Clinical Analyzer	ACE and NExCT Clinical Chemistry Systems
Reaction Type	Endpoint	Endpoint
Sample Type	Serum or heparin plasma	Serum
Sample Volume	5 μL	3 μL
Calibration	Each lot is calibrated by the manufacturer prior to shipment using material traceable to β-Quantification Reference Method (CDC). The 2-D barcode printed on each cartridge provides the analyzer with lot-specific calibration data.	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the standard.

Comparison Table		
Item	Device (k091333)	Predicate (k991733)
Use of Controls	Two levels of control per day	Two levels of control per day
Linearity Range	11 to 396 mg/dL	3 to 800 mg/dL
Detection Limit	1 mg/dL	3 mg/dL
Reagent Stability	Low Density Lipoprotein Cholesterol cartridges are stable until the expiration date on the box labels when stored at 2-8°C	Unopened LDL reagent is stable until the expiration date shown on the box and bottle labels when stored at 2-8°C
Testing Environment	Clinical laboratories or physician office laboratories	Clinical laboratories or physician office laboratories

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

1. CLSI EP6-A – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach (2003)
2. CLSI EP7-A2 – Interference Testing in Clinical Chemistry (2005)
3. CLSI EP5-A2 – Evaluation of Precision Performance of Quantitative Measurement Methods (2004)
4. CLSI EP10-A3 – Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures (2006)
5. CLSI EP9-A2 – Method Comparison and Bias Estimation Using Patient Samples (2002)
6. CLSI EP17-A – Protocols for Determination of Limits of Detection and Limits of Quantitation (2004)

L. Test Principle:

The single-use LDL reagent plastic container is composed of a bi-reagent cartridge holding two stable reagents, a reaction cavity, and a bar code label. The test is based on a photometric measurement of the amount of LDL present after other non-LDL cholesterol is solubilized by a detergent and then consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. LDL is then solubilized by a second detergent in the presence of a chromogenic peroxidase substrate to form a purple-red color. The amount of color formed, determined by measuring the increase in absorbance bichromatically at 546/660 nm on the S40 Clinical Analyzer, is directly proportional to the LDL cholesterol concentration.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

In-house precision studies were conducted as follows: three serum samples (with normal, intermediate, and elevated levels) were tested for LDL on one S40 Clinical Analyzer two times per run, two runs per day, for a total of 22 days. The mean, standard deviation, and % coefficients of variation (CV) were calculated for each sample.

Sample 1 Mean = 36 mg/dL LDL	Within Run	Between Run	Between Day	Total
Standard Deviation, mg/dL	0.8	0.3	0.3	0.9
Coefficient of Variation	2.2%	1.0%	0.8%	2.5%

Sample 2 Mean = 186 mg/dL LDL	Within Run	Between Run	Between Day	Total
Standard Deviation, mg/dL	2.3	3.7	0.0	4.4
Coefficient of Variation	1.3%	2.0%	0.0%	2.4%

Sample 3 Mean = 337 mg/dL LDL	Within Run	Between Run	Between Day	Total
Standard Deviation, mg/dL	4.0	5.4	2.8	7.3
Coefficient of Variation	1.2%	1.6%	0.8%	2.2%

Precision Studies were also conducted at three Physician Office Laboratories (POL). Three serum samples (with normal, intermediate, and elevated levels) were tested for LDL on three S40Clinical Analyzers (one at each lab) three times per run, one run per day, for a total of 5 days. The mean, standard deviation, and % coefficients of variation (CV) were calculated for each sample.

Lab	Sample	Mean	% CV or SD (mg/dL)	
			Within-Run	Total
POL 1	1	41	1.2 SD	1.2 SD
			2.8%	2.8%
POL 2	1	41	0.5 SD	1.0 SD
			1.1%	2.4%
POL 3	1	38	0.4 SD	0.5 SD
			1.0%	1.4%

POL 1	2	189	1.6 SD	2.4 SD
			0.9%	1.3%
POL 2	2	186	2.7 SD	2.7 SD
			1.5%	1.5%
POL 3	2	180	1.4 SD	2.1 SD
			0.8%	1.2%
POL 1	3	358	5.6 SD	7.1 SD
			1.6%	2.0%
POL 2	3	352	6.0 SD	6.5 SD
			1.7%	1.8%
POL 3	3	339	5.5 SD	6.7 SD
			1.6%	2.0%

b. *Linearity/assay reportable range:*

Commercial linearity standards at 11 different levels with values ranging from 11 to 396 mg/dL were used to determine linearity across the assay range. The mean value of each set of quadruplicate determinations was determined. Percent recoveries ranged from 90-102%. The linear regression equation obtained for the study was $y = 1.011x - 4.25$, $r^2 = 0.9996$.

The LDL assay reportable range using the S-Test LDL Cholesterol reagent cartridge on the S40 Clinical Analyzer is 11 to 396 mg/dL. If a sample is less than 11 mg/dL, the result is flagged by the analyzer as < 11 mg/dL-Range. If a sample result exceeds 396 mg/dL, the result is flagged by the analyzer as > 396 mg/dL-Range.

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

Each lot of S-Test LDL Cholesterol reagent cartridges is factory calibrated and traceable to the β-Quantification Reference Method. The 2-D barcode printed on each cartridge provides the S-40 Clinical Analyzer with lot-specific calibration data. This method has not been tested or certified by the Cholesterol Reference Method Laboratory Network.

Stability of reagent cartridges was determined at 2-10°C using real-time studies. Accuracy and precision data substantiate the stability claim of 12 months when stored at 2-8°C.

d. *Detection limit:*

The limit of detection was determined by assaying five low samples (serum samples) and five true blanks (human serum albumin in saline). Testing was carried out over three days on two S40 Clinical Analyzers. Both, the low samples and true blanks, were run every day for a total of replicates n=60. The limit of detection was determined to be 1 mg/dL. The assay reportable range is 11 to 396 mg/dL on the S40 Clinical Analyzer. If a sample is less than 11 mg/dL, the result is flagged by the analyzer as < 11 mg/dL-Range.

e. *Analytical specificity:*

Interference studies to determine the effect of Ascorbic Acid, Unconjugated Bilirubin, Hemolysis, and Triglycerides were performed. Serum pools containing normal and abnormal LDL cholesterol were spiked with various concentrations of ascorbic acid, unconjugated bilirubin, and hemoglobin as described in table below.

Interference	Normal LDL-C (mg/L)	Abnormal LDL-C (mg/L)
Ascorbic Acid (serum: 1.6 – 50 mg/dL)	39	140
Unconjugated Bilirubin (serum: 1.6 – 50 mg/dL)	120	142
Hemoglobin (serum: 31 – 1000 mg/dL).	120	142

Triglycerides (300 – 1170 mg/dL) were evaluated using four lipemic serum samples. All samples were tested in triplicate on the S40 Clinical Analyzer. Results from interference studies are described in the device labeling as follows. Ascorbic Acid and Bilirubin showed no significant interference at the tested conditions. No significant interference from hemolysis was detected for the normal sample. Hemoglobin concentrations greater than 500 mg/dL may cause interference. Negative interference occurred at 1000 mg/dL on the abnormal sample. Triglycerides showed no significant interference below 589 mg/dL. Triglyceride concentrations greater than 589 mg/dL may cause interference. Negative interference occurred at 1170 mg/dL triglycerides.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A series of 110 serum specimens with LDL cholesterol values ranging from 11 to 388 mg/dL were assayed on the S40 Clinical Analyzer using the S-Test LDL Reagent and the ACE Clinical Chemistry System using the ACE LDL-C Reagent as the reference method. Least-squares regression analysis yielded the following results:

Regression Equation	y = 0.965x + 0.85
Correlation Coefficient	0.9958
Std. Error Est.	6.57
Confidence Interval Slope	0.948 to 0.982
Confidence Interval Intercept	-1.73 to 3.42

Point of Care in Physician's Office Laboratories

Performance for the S-Test LDL cholesterol reagent was evaluated at three Physician Office Laboratories (POL). Operators assayed > 40 serum samples on the S40 Clinical Analyzer and the ACE Clinical Chemistry System. The following linear regression data was obtained:

POL	1	2	2
n	49	46	49
Range	12-348	16-348	13-322
Regression Equation	y = 0.939x + 7.95	y = 0.922x + 8.93	y = 0.936x - 0.92
Correlation Coefficient	0.9945	0.9953	0.9968
Standard Error	7.77	8.03	5.95
Confidence Interval Slope	0.910 to 0.967	0.895 to 0.949	0.914 to 0.959
Confidence Interval Intercept	3.58 to 12.32	4.28 to 13.58	-4.56 to 2.72

b. Matrix comparison:

A study was performed by assaying LDL cholesterol determinations on 34 paired samples drawn from the same patients in serum and lithium heparin plasma tubes at a clinical laboratory. All specimens were assayed on the S40 Clinical Analyzer using S-Test LDL cartridges. The serum results ranged

from 13 to 350 mg/dL. Least-square regression analysis yielded the following results (serum x-axis; plasma y-axis):

Regression Equation	y = 0.998x - 0.81
Correlation Coefficient	0.9990
Std. Error Est.	3.61
Confidence Interval Slope	0.982 to 1.014
Confidence Interval Intercept	-3.10 to 1.48

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 S-Test LDL cholesterol reagent uses the LDL reference range recommendations on lipid testing and management published in the "Third Report of National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Cholesterol in Adults (Adult Treatment Panel III); Final Report.

LDL Cholesterol (mg/dL)	
< 100	Optimal
100-129	Near optimal/above optimal
130-159	Borderline High
160-189	High
$\geq$ 190	Very High

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.